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APPLICATION AND TIMING EFFECTS OF QOI AND DMI FUNGICIDES AND A FOLIAR FERTILIZER ON OVERALL PLANT HEALTH AND GRAIN YIELD IN CORN

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APPLICATION AND TIMING EFFECTS OF QOI AND DMI FUNGICIDES AND A FOLIAR FERTILIZER ON OVERALL PLANT HEALTH AND GRAIN YIELD IN CORN

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Dedicated to my wife, Amber.

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ABSTRACT

Geis, Jason Phillip. M.S., Purdue University, May 2014. Application and Timing Effects of QoI and DMI Fungicides and a Foliar Fertilizer on Overall Plant Health and Grain Yield in Corn. Major Professors: James Camberato and Robert Nielsen.

The use of fungicides on corn has recently increased as a result of higher grain market prices, changes in cropping practices, higher disease incidence and severity, and the availability and marketing claims of new fungicides. Some of the marketed potential "plant health™" benefits include improved tolerance to drought and heat, improved N utilization, and increased stalk strength. Previous studies have displayed delayed canopy senescence, changes in water use efficiency, and reduced ozone damage in controlled environments. Foliar fertilization has also increased in popularity in recent years due to an increase in grain prices, manufacturer claims, and product availability.

Large-scale field experiments were conducted at three locations in 2012 and five locations in 2013 representing different soil types and growing conditions to evaluate the effectiveness of treatments and timing (V5, R1, V5+R1) of two QoI fungicides and two fungicides containing both QoI and DMI active ingredients. Foliar fertilizer containing N and B was applied at the V5 growth stage. Multiple measurements were conducted throughout the growing season to determine the effects of several common foliar

fungicides and a foliar fertilizer on different indicators of plant health. Measurements included disease assessments, leaf surface temperature, crop canopy reflectance, chlorophyll meter values, green leaf area below the earleaf, and stalk strength assessments. At the end of the growing season, grain yield and moisture content were estimated with a commercial harvester and calibrated yield monitor and grain was analyzed for nutrient concentration.

The foliar fertilizer had essentially no effect on the plant parameters measured and no effect on grain yield at any of the locations. The most consistent effect of foliar fungicides on the plant parameters measured was disease control. No fungicide effects were observed on leaf surface temperature or crop canopy reflectance. Small and inconsistent effects were observed with foliar fungicide applications made at R1 on chlorophyll meter values, the number of green leaves below the earleaf, and stalk strength (push test). Fungicides applied at V5 increased grain yield 13% of the time, and fungicides applied at R1 increased grain yield 50% of the time. Overall, the effects of fungicide applications at R1 were greater than those from applying fungicides at V5, and two applications of fungicides did not affect overall plant health or grain yield more than one application made at R1. Based on these data from the 2012 and 2013 growing seasons, applications of foliar fertilizer or foliar fungicides at the V5 growth stage were not beneficial. Applying foliar fungicides at R1 inconsistently improved some indicators of plant health and grain yield.

CHAPTER 1. A REVIEW OF LITERATURE

1.1 Introduction

Corn (*Zea mays* L.) is the most widely grown grain crop in the United States, with about 38.6 million ha planted in 2013, followed by soybeans at 31 million ha, and wheat with 22.7 million ha, which comprises 70% of the total crop area planted in the United States (USDA-NASS, 2014). In Indiana, corn is the most widely grown crop from year to year making up approximately 50% of the total crop acreage (USDA-NASS, 2014). The vast acreage planted in corn and productive management practices make the United States the world's largest producer and exporter of corn, and corn grain accounts for about 12% of all United States agricultural exports (USDA-ERS, 2009). Most of the exported corn is used for feed, with lesser amounts being utilized for industrial and other food use (USDA-ERS, 2009). Corn is used almost equally for feed and industrial use in the United States due to an increasing demand for ethanol production (USDA-ERS, 2013).

Traditionally, fungicides have only been used on seed or specialty corn crops; however, fungicide use on dent corn has increased tremendously since 2007 (Wise and Mueller, 2011). Wise and Mueller (2011) reported that over 4 million ha of corn in the United States are now treated with foliar fungicides. The recent increase in fungicide use on corn is the result of multiple factors: new fungicides becoming available, increased

market price of corn, marketing claims of fungicide manufacturers, increased incidence and severity of foliar diseases, and changes in cropping practices (Wise and Mueller, 2011). Reduced tillage, no-till, and continuous corn cropping practices have increased in recent years that have contributed to more crop residue, higher levels of disease inoculum, and thus increased fungicide use (Wise and Mueller, 2011). Many fungicides were produced in 2005 in anticipation of the requirement to control the newly discovered disease soybean rust caused by *Phakopsora pachyrhizi* Syd. & P. Syd, however the disease never reached epidemic levels across the majority of the United States (Wise and Mueller, 2011). The authors suggested that fungicide manufacturers promoted the use of foliar fungicides on corn, in part, to aid in reducing the massive fungicide stockpile (Wise and Mueller, 2011).

Many foliar fungicide manufacturers currently promote early season fungicide applications to corn from the V4 to V8 growth stages of development in addition to the traditional timing of VT to R1 (Jardine and Ciampitti, 2013). One of the selling points of early applications of foliar fungicides to corn producers is that the fungicides can be combined and applied with post-emergence herbicides at very little cost (Jardine and Ciampitti, 2013). Manufacturers also promote the use of fungicides for reducing stress and improving overall plant health through physiological plant responses even in the absence of disease. Many producers now make "insurance" applications of foliar fungicides due to the market prices, company claims, and fungicide availability. Prophylactic fungicide use is of great concern with a single-site mode of action because the fungicides are at a higher risk for becoming ineffective due to the development of

resistant pathogens. Resistance has already developed for multiple fungicide modes of action in many fungal diseases in other crops (FRAC, 2013).

Foliar fertilization has increased in popularity in recent years due to the increase in corn prices. With higher corn prices, producers are willing to apply a variety of products if they believe it will improve their overall crop yields (Lentz, 2012). Fertilizer manufacturers have also begun to promote slow-release foliar fertilizers that are supposedly more efficient than the soil-applied forms (Sawyer, 2009). Some producers include foliar nutrients in their post-emergence herbicide tank mix to reduce trips across the field; however, university trials have not shown a consistent response to foliar-applied nutrients (Lentz, 2012). Solo foliar applications of macronutrients is not sufficient to meet plant demands, but foliar applications of micronutrients may be a viable option since they are needed in such low quantities (Lentz, 2012).

1.2 Corn Growth and Development

Understanding the growth and development of corn is critical in understanding how specific stresses, such as drought or foliar diseases, affect the plant. Timing of the stress largely affects how the plant will respond. All growth stages discussed are as described by Abendroth et al. (2011). The growth stage on a field scale is defined when 50% or more of plants are estimated to be at a certain stage (Abendroth et al., 2011). Corn plants have two different stages of development: vegetative and reproductive. During vegetative growth, the plant establishes, grows, produces photosynthetic leaf area, initiates all growing points, and determines the potential size of the ears in terms of ovule

numbers (Abendroth et al., 2011). The corn plant devotes almost all of its energy and photosynthate to grain fill during reproductive growth (Nielsen, 2008).

Ears and tillers are differentiated from axillary meristems starting shortly after emergence, beginning at the lowermost stalk node and continuing up the stalk until the harvestable ear is initiated around growth stage V5 (Lejeune and Bernier, 1996). The harvestable ear is typically found at nodes 12, 13, or 14 (Abendroth et al., 2001). At about the same time (V5), the tassel is initiated and the initial development of these two reproductive structures is sensitive to stress (Lejeune and Bernier, 1996). Severe stress may limit the potential ear size before pollination even occurs, depending on the growth stage of the crop at the onset of stress (Nielsen, 2007). The maximum number of kernel rows is set by approximately V7, but this is not likely to change due to stress, as row number is strongly influenced by the plant's genetics (Abendroth et al., 2011). The initiation of potential kernels row^{-1} begins at the base of the ear, continues toward the tip, and completes approximately one week prior to silk emergence (Cárcova et al., 2003). Stress can greatly influence potential kernels row^{-1} from approximately V7 through V15 (Nielsen, 2007; Abendroth et al., 2011). Each ear can develop up to 1,000 ovules, although there are typically only about 400 to 600 harvestable kernels ear^{-1} (Nielsen, 2010a). Due to early initiation of plant structures, stresses on the plant could negatively impact these reproductive structures before they are actually visible to the naked eye (Lejeune and Bernier, 1996).

The plant reaches its maximum height and number of fully developed leaves at or shortly after the VT growth stage and begins to redirect its photosynthate to grain fill (Nielsen, 2010b). Therefore, defoliation after this growth stage directly interferes with

overall yield at harvest by reducing leaf area present to produce photosynthate for grain fill (Nielsen, 2010b). Silking (R1) is the first stage of reproductive growth, and this period is the most sensitive to stress regarding the number of harvestable kernels ear^{-1} (Abendroth et al., 2011). Stress can affect the synchronization of pollen shed and silk emergence leading to poor pollination (Abendroth et al., 2011). Drought stress, in particular, often delays silk elongation and hastens pollen development. Ears are approximately 40 to 45% of their final length at R1 (Cárcova et al., 2003). Even if the ovule is fertilized, kernel abortion can occur during R2 (blister) and R3 (milk) if carbohydrate supply from the plant is inadequate (Abendroth et al., 2011). By R4 (dough), stress will not cause kernel abortion, but it can, however, decrease kernel weight by decreasing the amount of starch accumulation in the kernel (Abendroth et al., 2011). At the beginning of R5 (dent), approximately 45% of the final kernel weight has been accumulated (Abendroth et al., 2011). By half milk line during R5, over 90% of the final kernel weight has been accumulated (Afuakwa and Crookston, 1984; Ma and Dwyer, 2000). Once the plant develops the "black layer" at the R6 stage of development (physiological maturity), maximum dry matter accumulation has been achieved (Daynard and Duncan, 1969). Environmental stress after this time has no effect on final grain yield (Abendroth et al., 2011).

1.3 Plant Diseases and Management

1.3.1 General Information

The goal of any disease management practice is to reduce the amount of disease to a level below economic threshold (Maloy, 2005). The basic principles of disease

development and management, including the disease triangle, disease cycle, disease progress curve, and general control principles must be thoroughly understood to effectively and efficiently control disease. The ability of a pathogen to infect a host is dependent on the interaction between the pathogen, host, and environment, and it is depicted by the disease triangle (Agrios, 2005). The amount of disease that develops is dependent on the favorability of the environment specific to the pathogen, virulence and abundance of the pathogen, and susceptibility of the host to the particular pathogen (Agrios, 2005). Three options exist to decrease levels of disease: reduce the amount of inoculum, decrease the rate of infection, and/or reduce the amount of time the host is susceptible to infection (Arneson, 2001).

Understanding the disease life cycle is critical in determining which control methods to implement. The disease life cycle consists of production of inoculum, dissemination of inoculum, host inoculation and pre-penetration, penetration and ingress, infection, colonization and growth, and overseasoning of inoculum (Agrios, 2005). A variety of general disease control principles may be utilized to manage plant diseases including avoidance, exclusion, eradication, protection, resistance, or therapy (Maloy, 1993). Each of the principles disrupts a specific part of the disease cycle, so understanding the biology of the pathogen is important in order to effectively disrupt the "weak link" of the cycle (Maloy, 1993).

The fungicides in this study contain chemistries that provide both protective and some early-infection therapeutic properties (Mueller et al., 2013). Protective fungicides must be applied before the onset of disease in order to prevent the penetration and ingress of a pathogen (Maloy, 1993). Therapeutic or curative fungicides can be applied after the

onset of disease, thereby preventing infection, ingress of the pathogen, as well as providing some protective properties (Maloy, 1993).

Foliar diseases of corn have become an increasing problem in recent years, thus increasing the need for effective disease management techniques (Wise and Mueller, 2011). Reduced tillage and no-till cropping practices are becoming more common (USDA-ERS, 2010), but the additional crop residue provides a source of inoculum for many plant pathogens (Mueller et al., 2013). Foliar pathogens result in lesion formation or cause blighting of the photosynthetic tissue, making those areas nonfunctional, which reduces overall yield (Ward et al., 1999). Reducing the photosynthetic leaf area in corn can result in the transfer of carbohydrates from the stalk to the ear, which can indirectly lead to stalk rot and lodging (Rees and Jackson, 2008). Diseased plants tend to have increased transpiration rates due to the destruction of the cuticle, increase in the permeability of leaf cells, and dysfunction of stomata (Agrios, 2005). Respiration rates can also increase, which means that affected tissues can use up reserve carbohydrates at a higher rate than healthy tissues (Agrios, 2005).

1.3.2 Gray Leaf Spot

The most common foliar disease of corn in the United States is gray leaf spot (GLS) caused by *Cercospora zeae-maydis* Tehon and Daniels (Mueller et al., 2013). The fungus survives and overwinters within infected corn crop residue on the soil surface (Ward et al., 1999). Following periods of warm weather (25-30˚C) and high humidity (90% relative humidity), the fungus produces spores that are dispersed to the lower leaves by wind and/or rain (Ward et al., 1999; Wise, 2010). Spores are able to remain latent on

the leaf surface for extended periods of time, waiting on conducive environmental conditions for infection (Beckman and Payne, 1982). Under extended periods of high relative humidity, germ tubes extend and penetrate through plant stomata in response to tropistic attractions (Beckman and Payne, 1982). Symptoms may take up to two weeks after infection to appear on the lower leaves (Wise, 2010). Each lesion can produce many more spores, which can be splashed or blown upward to leaves higher in the plant canopy that can lead to additional blighting and necrosis (Ward et al., 1999; Wise, 2010). Neither the plant age nor the leaf age influence susceptibility of plants to infection (Beckman and Payne, 1982). Lesions typically appear gray to tan in color, rectangular in shape, and run parallel with leaf veins (Ward et al., 1999). However, on resistant hybrids, lesions tend to stay small and have a round or jagged shape (Wise, 2010).

1.3.3 Northern Corn Leaf Blight

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* (Pass.) is another common disease of corn in the Midwest (Perkins, 1987). The fungus overwinters within infected corn residue on the soil surface as mycelia and conidia (Levy and Pataky, 1992). Moderate temperatures (20-25˚C), high humidity, and a dew period of about 6 to 18 hours are required for sporulation. Spores are splashed or wind-blown onto corn leaves, and they are typically found in the lower and middle third of the plant canopy (Levy and Pataky, 1992; Wise, 2011). Lesions may form 7 to 12 days after infection depending on environmental conditions (Wise, 2011). Additional spores may be produced within each lesion and infect other parts of the plant (Wise, 2011). Symptoms appear as oblong tan or grayish lesions that form parallel to leaf margins (Wise, 2011).

1.3.4 Anthracnose Leaf Blight

Anthracnose caused by *Colletotrichum graminicola* (Ces.) is common in the Midwest throughout the growing season and can cause leaf blight, top-dieback, or stalk rot (Bergstrom and Nicholson, 1999). The fungus overwinters as saprophytes on corn residues at the soil surface (Bergstrom and Nicholson, 1999). Penetration occurs when temperatures range from about 25-30˚C during overcast days with high humidity (Bergstrom and Nicholson, 1999). Initial infection of the leaf blight phase occurs once spores are splashed onto seedling leaves from the crop debris (Bergstrom and Nicholson, 1999). Symptoms appear on the lowest leaves first as tan or brown oval shaped lesions with a purplish margin (Robertson, 2007).

1.3.5 Effects of Foliar Fungal Diseases

Lesions produced by foliar fungal diseases reduce the amount of overall photosynthetically active leaf tissue in a plant, thus reducing production of photosynthate and decreasing grain yield when severity is high (Ward et al., 1999). Percentage of overall yield loss is largely affected by the timing of disease development and location in the plant canopy (Levy and Pataky, 1992; Stuckey et al., 1993). Reductions in grain yield have been observed when onset of disease occurs near R1 and continues beyond 2-3 weeks after pollination (Levy and Pataky, 1992). Grain yield reduction is minimal when little to no blighting occurs until 6 weeks after silking (Stuckey et al., 1993). Studies have shown that the upper 8-10 leaves of a corn plant contribute to almost 90% of grain

fill (Adee et al., 2005; Allison and Watson, 1966). Loss of leaf tissue from the lower third of plant due to blighting has very little to no effect on grain yield (Levy and Pataky, 1992).

Yield loss is negligible until a certain threshold is reached. In a study on sweet corn inoculated with *Exserohilum turcicum*, no yield difference was observed until severity of NCLB was > 8% in the upper 75% of the crop canopy at fresh market maturity (20 days after mid silk) (Pataky, 1992). Once this threshold was reached, the author found reductions in yield to be about 0.4%-1% for each 1% increase in severity (Pataky, 1992). Patrick Lipps (1998) found 0-5%, 6-25%, 25-75%, and 75-100% severity of GLS on the earleaf at R5 to decrease yield by 0-2%, 2-10%, 5-20%, and 15-50%, respectively (Wise, 2010). Multiple studies have shown that decreases in grain yield due to foliar diseases are primarily attributable to decreased kernel weight (Perkins, 1987; Raymundo, 1981; Rees and Jackson, 2008; Ward et al., 1999). Bowen and Pedersen (1988) saw a reduction in kernel number when disease severity was high during early stages of grain fill.

1.3.6 Stalk Rot

Foliar diseases can indirectly increase the amount of stalk rot when disease severity is high by reducing photosynthetically active leaf tissue (Dodd, 1980; Mortimore and Ward, 1964). Disease, low soil moisture, low light intensity, high plant density, and other environmental stresses influence the plant's ability to produce carbohydrates required for grain fill and maintenance (Dodd, 1980; Mortimore and Ward, 1964). During grain fill, the carbohydrate requirements for kernels are fulfilled first (Mortimore

and Ward, 1964). Carbohydrates are translocated to each kernel at a fairly constant rate, with about 20% of the grain weight coming from carbohydrates stored in the stalk (Dodd, 1980). Light intensity, effective leaf area, moisture availability, and mineral concentration can affect the ability of plants to meet grain fill requirements and the amount of carbohydrates taken from the stalk (Dodd, 1980).

1.3.7 Disease Management

Multiple disease management options exist for foliar fungal diseases. Implementing multiple disease management practices each season is the most effective way to keep disease levels low. Many of the most common foliar diseases in the Midwest overwinter on corn residue at the soil surface (Levy and Pataky, 1992; Lipps, 1983; Ward et al., 1999). Crop rotation and tillage are very effective at reducing levels of primary inoculum (Stuckey et al., 1993; Ward et al., 1999). Selecting hybrids with disease resistance is one of the simplest and most effective methods available for reducing infection levels (Perkins, 1987; Stuckey et al., 1993; Ward et al., 1999). Fungicides are effective at controlling fungal diseases, but timing and frequency of application is crucial to effectiveness (Ward et al., 1997). The major limitation in making fungicide application decisions is the inability to predict disease severity and the potential yield impact of the disease (Munkvold et al., 2001). In a study on frequency and timing of fungicide applications for management of GLS, the authors determined the optimum time to treat was when disease levels were about 2 to 3% on the lowermost five leaves of the plant, regardless of growth stage (Ward et al., 1997).

1.4 Fungicide Background

1.4.1 Nomenclature

Fungicides have three different names that can be found on the product label: chemical name, common name, and trade name (Latin, 2011). Internationally sanctioned authorities assign the chemical and common name to pesticides (Latin, 2011). The chemical name (i.e. [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester) is used to determine the chemical compounds that make up the active ingredient, and the common name (i.e. pyraclostrobin) simplifies the chemical name making it more useful (Latin, 2011). Finally, the trade name, such as Headline®, is the patented name assigned by the manufacturer (Latin, 2011; Mueller, 2006b).

1.4.2 Mode of Action/Resistance

The mode of action refers to how the active ingredients interfere with fungal growth (Latin, 2011). The active ingredients in multi-site fungicides disrupt multiple metabolic processes in fungal cells, making them less prone to resistance development (Latin, 2011). Active ingredients in site-specific fungicides are much more susceptible to the development of resistance because of the interference with a particular biochemical process in fungi (Latin, 2011). Multiple modes of action have been identified over the years. The Fungicide Resistance Action Committee (FRAC) has classified all commercial fungicides according to their mode of action in order to reduce development of resistance (FRAC, 2007).

These include (FRAC, 2007):

Group A → Nucleic Acid Synthesis

Group B → Microtubules, Mitosis, Cell Growth

Group C → Mitochondrial Respiration

Group D → Amino Acids, Protein Synthesis

Group E → Signal Transduction

Group F → Lipids, Membrane Synthesis

Group G → Sterol Biosynthesis

Group H → Glucan Synthesis

Group I → Melanin Synthesis

Group P → Host Plant Defense Induction

Group U → Unknown

Group M → Multi-site

To reduce the risk of resistance, it is best to use fungicides with different or multiple modes of action (Mueller, 2006a). To reduce the risk of fungal resistance, fungicides listed within the same FRAC grouping should be alternated or mixed with other fungicide groups, if available (Mueller, 2006a).

1.4.3 Role in Protection

Preventative activity, early-infection activity, anti-sporulant activity, and eradication are types of protection obtained by the use of fungicides (Mueller, 2006b). Preventative activity occurs when a fungicide is applied prior to infection by fungi (Mueller, 2006b). Fungicides with early-infection activity can be applied shortly after

infection begins to prevent further damage to the plant (Mueller, 2006b). Anti-sporulent fungicides reduce the amount of inoculum available to infect other plants by preventing spore production, but they do not prevent lesion expansion of the current disease (Mueller, 2006b). Eradication refers to stopping disease development after symptoms develop. Very few fungicides have the ability to eradicate pathogens, and this is not a reliable means of disease control (Mueller, 2006b). Some fungicides contain a mixture of active ingredients that provide a variety of protective activities within a single product.

1.4.4 Mobility

Mobility refers to the ability of the active ingredient to move, or not move, within the plant (Latin, 2011). Contact fungicides are immobile and only affect fungi present on the plant surface, where penetrant fungicides are absorbed into underlying plant tissue (Latin, 2011). Complete coverage is essential in effectiveness of contact fungicides, as these fungicides do not protect parts of the plant not contacted by the fungicide (Latin, 2011). However, contact fungicides may redistribute on the plant with water, such as rain, dew, or irrigation (Latin, 2011). With adequate coverage, one can expect 5-50% of penetrant fungicides to be absorbed into the plant (Latin, 2011). Because penetrant fungicides have high solubility in the plant, they are more effective at controlling root pathogens (Latin, 2011). Penetrant fungicides can be classified into three categories based on movement within the plant: acropetal, local, and systemic (Latin, 2011). The active ingredient moves upward or outward in the plant via apoplastic movement within the xylem in acropetal penetrants (Latin, 2011). Local penetrants are mobile for short distances only, and generally move via apoplastic movement to the opposite side of

leaves (Latin, 2011). The most mobile penetrant fungicides are the ones that can move systemically, translocating up or down via symplastic movement within the phloem (Latin, 2011).

1.4.5 Effectiveness

Many factors are involved in fungicide efficacy against disease. First of all, application timing, disease severity, and environmental conditions should be considered before making a fungicide application (Mueller et al., 2013). Application timing and disease threshold must be considered for each crop-pathogen combination (Mueller et al., 2013). Environmental conditions conducive for disease increase the chances of benefitting from a fungicide application, if the fungus can be managed through fungicides (Mueller et al., 2013). One of the most important rules when making an application is to follow the product label (Mueller, 2006d). Accurate disease diagnosis is important in selecting which fungicide to use for control (Mueller, 2006d). Using a product that is less than two years old ensures the active ingredient is still effective (Mueller, 2006d). When mixing the product, it is important that the pH of the water is within the recommended range for that particular product (Mueller, 2006d). Sprayer calibration is very important to ensure proper nozzle type, droplet size, rate, pressure, and speed are used in order to maximize coverage (Mueller et al., 2013). Finally, allowing enough time for the product to dry before a rain is essential in effectively controlling disease (Mueller, 2006d).

1.4.6 History

This section is a summarized version of an extensive review conducted by P.E. Russell (2005) over the history of fungicide development. Disease control in plants has come a long way in the last century. In the late 19th century, individuals started to utilize chemicals for disease control. Many of the early "fungicides" contained sulfur. The Bordeaux mixture, discovered by Millardet, was one of the best known early fungicides, and it contained copper sulfate and lime. By the start of the 20th century, mycology and plant pathology were both well-known areas of study. In this era, rules and regulations on safety were nonexistent, and most fungicides were homeade. Many fungicides in this era resulted in poor disease control and required high rates of active ingredients in the 10-20 kg ha^{-1} range.

Chemical disease control made great advances after World War II. Some of the chemical resources developed during war were used in agricultural research. Reliance on homemade fungicides declined as commercially developed products became available. Research focus switched from epidemiology to physiological aspects of plant infection.

Chemical crop protection expanded rapidly in the 1960's. Methyl benzimidazole carbamate fungicides were distinguished from other fungicides developed thus far due to systemic properties. Also developed in this time period was the chlorothalonil, dodemorph, ethirimol, and carboxin fungicides. The active ingredients were effective for control against Deuteromycetes, Ascomycetes, and Basidiomycetes.

During the 1970's, fungicide application systems improved. The rates at which fungicides were applied drastically decreased. The new fungicides were generally developed with a single site of action, however, and resistance began to appear. The

Fungicide Resistance Action Committee (FRAC) was developed in the early 1980's to help manage fungicide resistance development. Many of the fungicides we use today were developed after the 1970's. These include the DMI fungicides, QoI fungicides, and others.

1.5 QoI Fungicides

1.5.1 Mode of Action

Quinone outside Inhibitors, or QoI – fungicides (FRAC Code 11), inhibit mitochondrial respiration by binding to a specific target site (FRAC, 2007). Once applied to the crop, these fungicides act by binding to the quinol oxidation site of cytochrome b, located in the cytochrome bc_1 complex in the inner mitochondrial membrane of fungi (Balba, 2007; Bartlett et al., 2002; Köhle et al., 2002). Binding to cytochrome b inhibits the transfer of electrons from cytochrome b to cytochrome c in fungi, which stops reduced nicotinamide adenine dinucleotide (NADH) oxidation and adenosine triphosphate (ATP) synthesis (Balba, 2007; Bartlett et al., 2002; Köhle et al., 2002). This halts energy production in fungi and ultimately leads to death (Balba, 2007).

Some fungicides within the QoI group are classified as strobilurin fungicides. These fungicides are weak systemic fungicides or are locally systemic, meaning they are not freely mobile in the plant and coverage on the plant is essential for efficacy (Balba, 2007). Once applied, the droplet will spread out on the leaf surface and is absorbed by the cuticle of the leaf (Balba, 2007). Some of the active ingredient moves to the cuticle on the other side of the leaf, and this is known as translaminar movement (Balba, 2007). Strobilurins have a residual period of approximately 21 days, and applications are

typically made for protectant, preventative, or curative properties (Balba, 2007). Strobilurins are very effective at preventing fungal spore germination, but have almost no effect on fungal mycelium (Balba, 2007).

1.5.2 Use in Field Crops

QoI fungicides are currently one of the most effective fungicide groups used to control disease in field crops, but studies have shown inconsistent effects on plants induced by application of strobilurin fungicides. Some studies have found a consistently greater yield increase from strobilurin-based fungicide programs compared to demethylation inhibiting (DMI)-triazole-based fungicide programs, even when both fungicide groups controlled disease similarly (Bartlett et al., 2002). Triazole fungicides are classified as demethylation inhibitors, and they inhibit the C14-demethylase enzyme in fungi which is essential for membrane structure and function (Mueller, 2006c). Abnormal fungal growth eventually leads to death, but triazoles do not inhibit spore germination (Mueller, 2006c). The greater yield response of strobilurin fungicides has been associated with the strobilurin "greening effect", whereby treated plants maintain green leaf area longer in the growing season than non-treated plants (Bartlett et al., 2002). Two general hypotheses have been proposed to explain the maintained green leaf area (Bartlett et al., 2002). Application of these products may maintain green leaf area due to non-disease related physiological effects on the plant, thus increasing yield (Bartlett et al., 2002). Secondly, the fungicidal property of strobilurins prevents spores from germinating, unlike triazoles, which may prevent the host plant from initiating defense mechanisms, resulting in green leaf area retention and increased yield (Bartlett et al.,

2002). The impact of fungicides within the QoI class depends on a variety of factors, some of which include the crop species, environmental factors, disease pressure, and application equipment. A study conducted on winter wheat to assess the effect of triazole and strobilurin fungicide applications, demonstrated a significant interaction between fungicide and wheat variety as well as fungicide application timing (Pepler et al., 2005). This study indicates the variability of responsiveness to a fungicide even when the same active ingredient was used. Knowing specifics about the variety of crop and determining proper application timings is crucial in obtaining a beneficial response to fungicides. Past studies have shown a variety of plant responses to strobilurin fungicides, including disease control and physiological changes in plants induced by the active ingredient (Köhle et al., 2002). A supplemental label (EPA Reg. No. 7969-186) claims that application of the strobilurin fungicide pyraclostrobin (Headline®; BASF Corporation, Research Triangle Park, NC) provides improved plant health and tolerance to environmental stresses, such as drought, heat, cold, and ozone damage (BASF, 2008). This supplemental label (EPA Reg. No. 7969-186) also states that application can provide "improved utilization of nitrogen…., improved stalk strength and better harvestability, induced tolerance to stalk diseases, better tolerance to hail, and more uniform seed size" (BASF, 2008). Multiple university studies have shown some of these marketing claims to be inconsistent. One study indicated that stalk rot severity was not improved by the application of pyraclostrobin at the VT/R1 growth stage under low levels of disease (<5% severity on earleaf at R4) at two of four locations (Byamukama et al., 2013). Another study with simulated hail damage in corn indicated that application of pyraclostrobin (Headline®) or azoxystrobin (Quadris®; Syngenta Crop Protection, Inc., Greensboro, NC)

resulted in significantly lower levels of GLS in one year of the study, but fungicide application had no effect on disease severity during the second year of the study (Bradley and Ames, 2009). Also noted was that simulated hail damage increased disease levels from 44% in non-damaged plots to 56% in damaged plots in one year of the study, but not the second year. Fungicides had no effect on yield in either year (Bradley and Ames, 2009). The authors concluded that predictors of disease pressure, such as surface residue, planting date, maturity, and hybrid resistance should be criteria used for making decisions regarding fungicide application rather than hail damage. Very few field studies have been conducted on corn with regards to the potential effects of strobilurin fungicides on hormonal effects, N accumulation, oxidative resistance, or yield components.

1.5.3 Fungicide Application

Product labels (Section 3) for the four fungicides included in this study are very similar regarding application timings and methods (EPA Reg. No. 7969-186 and 7969-291, BASF Corporation, Research Triangle Park, NC; EPA Reg. No. 100-1098, Syngenta Crop Protection, Inc., Greensboro, NC; EPA Reg. No. 264-1093, Bayer CropScience LP, Research Triangle Park, NC). Pyraclostrobin (Headline®), azoxystrobin (Quadris®), prothioconazole plus trifloxystrobin (Stratego YLD®), and pyraclostrobin plus metconazole (Headline AMP®) can be applied to crops via ground sprayer, aerial equipment, or through irrigation equipment. The fungicide labels specify that "sufficient water volume for adequate coverage and canopy penetration" is needed to ensure optimum disease control. For example, the label for Headline AMP® recommends at least 94 L ha^{-1} of water for ground application. Adjuvants may not be used after growth

stage V8 and prior to growth stage VT when applying Stratego YLD or Headline AMP. The Stratego YLD label also specifies that application is not recommended when corn is under severe environmental stress conditions. To limit resistance development potential by the target diseases, no more than two sequential applications of any of the four products may be made without alternating modes of action. Headline and Headline AMP are to be applied prior to disease development, and Quadris and Stratego YLD are to be applied at the onset of disease.

BASF Corporation has developed a corn fungicide solution guide to maximize disease control and plant health in corn. Applications of Headline fungicide applied at V5 toV8 are reported to increase yields by 250 to 500 kg ha^{-1} on average by controlling disease, improving plant health, increasing photosynthesis, decreasing respiration, and increasing N efficiency (BASF, 2012). Applications of Headline AMP between VT and R3 are said to increase yields by 750 to 1000 kg ha^{-1} on average by controlling disease, improving plant health, increasing photosynthesis, increasing stalk strength, and minimizing drought stress (BASF, 2012). According to BASF, sequential applications provide the most benefit under field conditions with high plant populations, continuous corn, high residue, history of disease, and disease-sensitive hybrids (BASF, 2012).

1.5.4 Nitrogen Accumulation

Strobilurin fungicides can potentially affect the amount of N accumulation in corn grain and leaves, but results are not always consistent between controlled laboratory research and field research. For example, after the application of pyraclostrobin on hydroponically grown wheat (*Triticum aestivum* L.) plants, nitrite and ammonia

accumulated in greater amounts in the leaves compared to the untreated control during the first night and persisted 3 nights after application (Köhle et al., 2002). This accumulation was the result of an enhancement of nitrate reduction during the night, which also led to a 20% increase in plant biomass over the untreated control two weeks after application (Köhle et al., 2002). The authors noted that the reduction of nitrate to nitrite is the rate-limiting step in N assimilation. A study conducted on spinach (*Spinacia oleracea* L.) leaf discs in buffer solution demonstrated an increase in nitrate reduction activity in both dark and light conditions when treated with kresoxim-methyl (Glaab and Kaiser, 1998). The authors concluded that kresoxim-methyl caused an additional activation of nitrate reductase due to acidification of the cytoplasm, but they stated that more work needed to be done in order to determine long-term effects under field conditions. In a different research study, the application of strobilurin and triazole fungicide mixtures on wheat in a field experiment increased the green area duration of the flag leaf, which corresponded with increases in grain N due to an extended period of accumulation of N (Ruske et al., 2003b). These authors concluded that the improvements in N uptake from the soil, remobilization of N from vegetative tissues after anthesis, and extended grain filling time was due to the delayed senescence of the flag leaf by controlling disease, and not due to the biochemical effects of the fungicide itself.

1.5.5 Hormonal Changes

Some studies show that strobilurin fungicides affect plant hormones, both indirectly through disease control, and directly through plant absorption of the active ingredient. Plants increase production of ethylene when subjected to a variety of abiotic

(temperature, drought) and biotic (pathogen, insect) stresses (Grossmann and Retzlaff, 1997; Morgan and Drew, 1997). Ethylene regulates developmental processes, including plant aging and reproductive growth (Grossmann and Retzlaff, 1997). When levels of ethylene are increased in the plant, leaf senescence accelerates and causes a premature ripening of the grain and a decrease in yield (Grossmann and Retzlaff, 1997). Cytokinins are enhancers of light-induced chlorophyll and thylakoid formation in plants (Grossmann and Retzlaff, 1997). Ethylene tends to accelerate the breakdown of cytokinins, so when levels of ethylene are increased, there are lower levels of cytokinins in the plant (Grossmann and Retzlaff, 1997). Abscisic acid (ABA) plays an important role in regulating stomatal behavior and gas exchange in drought-stressed plants by promoting stomatal closure (Wilkinson and Davies, 2002). Dehydration of plant cells, both root and leaf, stimulates production of ABA to reduce the amount of water lost through transpiration, which is related to the water use efficiency (WUE) of the plant (Wilkinson and Davies, 2002).

Wheat plants grown in growth chambers had increased concentrations of cytokinins in the shoot and a decrease in 1-aminocyclopropane-1-carboxylic acid (ACC) concentrations, which is the precursor of ethylene in biosynthesis, after the application of kresoxim-methyl; consequently reducing the loss of chlorophyll and delaying plant senescence (Grossmann and Retzlaff, 1997). The authors concluded that kresoxim-methyl affected the rate of conversion of S-adenosylmethionine to ACC, thus reducing production of ethylene under stressful conditions (Grossmann and Retzlaff, 1997).

Abscisic acid levels were found to increase 2-fold when kresoxim-methyl was applied to wheat plants grown in growth chambers, which reduced stomatal opening and

reduced water consumption by 8% due to reduced transpiration rates compared to the controls (Grossmann et al., 1998). A field experiment conducted on grapevines (*Vitis vinifera* L.) showed a very brief increase in ABA content when pyraclostrobin was applied, but this fungicide-induced ABA was diluted throughout the day due to the transpiration flux (Diaz-Espejo et al., 2012). Application of a strobilurin on grapevines in the field had little effect on leaf gas exchange and plant water status as influenced by ABA content and stomatal conductance (Diaz-Espejo et al., 2012). Treatments of picoxystrobin, pyraclostrobin, azoxystrobin, kresoxim-methyl, and trifloxystrobin reduced the rate of conductance of water through stomata, transpiration rates, net photosynthesis, and intercellular carbon dioxide concentration in wheat, soybean (*Glycine max* Merr.), and barley (*Hordeum vulgare* L.) grown in a greenhouse compared to the control and treatment with a triazole fungicide (Nason et al., 2007). Application of pyraclostrobin, azoxystrobin, and trifloxystrobin reduced the amount of water required to fix 1 mole of carbon dioxide under well-watered conditions, thus increasing WUE relative to the well-watered control. However, the WUE of plants treated after being subjected to water-deficit conditions decreased (Nason et al., 2007). When transpiration rates were already low due to stomatal closure in the water-stressed plants, reducing transpiration rates even more due to application of strobilurin fungicides had an even more negative impact on net photosynthesis (Nason et al., 2007). Treatments of pyraclostrobin on four wheat genotypes was found to decrease water uptake compared to the control when grown in a controlled environment, but no effects were observed under field conditions (Inagaki et al., 2009). Effects are more difficult to measure in field conditions due to unpredictable environmental factors and different soil types.

1.5.6 Senescence and Green Leaf Duration

Leaves show delayed senescence and thus longer duration of green leaf area after strobilurin fungicides are applied due to disease control and possible hormonal changes in the plant. A field experiment conducted on winter wheat showed that applications of epoxiconazole and azoxystrobin were both effective at controlling levels of septoria leaf blotch (*Septoria tritici* Rob. Ex Desm.) and delaying senescence (Ruske et al., 2003a). Two applications of azoxystrobin delayed senescence longer than one application, and azoxystrobin delayed senescence longer than triazole-only applications (Ruske et al., 2003a). In conclusion, the authors noted that a strobilurin fungicide did maintain green leaf area, but the greatest responses were seen on the most susceptible cultivars and years with high disease pressure. A similar study on wheat showed that azoxystrobin delayed green leaf area decline compared to control, and the effects were more pronounced on susceptible hybrids during years of high disease pressure (Gooding et al., 2000). The green leaf area on the flag leaf on multiple varieties of wheat could be extended longer during wet summers than during dry summers after application of fungicides (Pepler et al., 2005). The duration of leaf life by the prevention of disease adequately explains the increases of grain yield after applications of fungicides rather than physiological effects of fungicides on plant metabolism (Dimmock and Gooding, 2002). The duration of green leaf life was found to be extended after application of strobilurin fungicides on grapevines, but the actual chlorophyll content was not increased due to application as evaluated by a SPAD meter (Diaz-Espejo et al., 2012).

Plants expend large amounts of energy as defense mechanisms against pathogens by increasing synthesis of callose, lignin, phytoalexins, and nucleic acids (Smedegaard-

Petersen and Tolstrup, 1985). This energy expenditure can lead to an increased rate of leaf senescence without any visible signs of disease (Smedegaard-Petersen and Tolstrup, 1985). Resistant barley was continuously inoculated with *Erysiphe graminis* f. sp. *hordei*, the causal agent of powdery mildew, throughout growth of the plants in a growth chamber experiment, and even though no disease was present, there was a significant decrease in grain yield, seed weight, and yield compared to the non-inoculated control due to plant defense reactions (Smedegaard-Petersen and Tolstrup, 1985). Saprophytes are present on living plant surfaces, but they are unable to infect green, growing plant tissue (Smedegaard-Petersen and Tolstrup, 1985). Even though saprophytes cannot actively infect plants, these organisms still seem to induce plant defense mechanisms leading to increased energy expenditure by the plant and an increased rate of leaf senescence (Bertelson et al., 2001; Smedegaard-Petersen and Tolstrup, 1985). Treatments of azoxystrobin on saprophyte-inoculated disease-free wheat in a glasshouse experiment proved that azoxystrobin does not directly influence senescence of plants through physiological changes, but azoxystrobin did prolong the duration of leaf life by inhibiting senescence-promoting activity of saprophytes (Bertelson et al., 2001). Azoxystrobin does not primarily reduce germination of spores, but it has been found to have inhibitory effects on development of fungal germ tubes, thus reducing plant defense mechanisms (Bertelson et al., 2001). The prolonged green leaf duration induced by strobilurins could be partially due to fewer defense reactions induced by the plant (Bertelson et al., 2001). Smedegaard-Petersen and Tolstrup (1985) conducted a similar experiment on barley and had similar findings.

1.5.7 Yield and Mean Grain Weight

Yield response of plants to fungicide applications is affected by multiple factors, some of which include disease pressure, hybrid susceptibility, environmental conditions, and timing of application (Henry et al., 2011; Paul et al., 2011; Ruske et al., 2003b). Physiological responses, disease control, and extended green leaf duration do not always contribute to significantly higher yield in the field since multiple factors are involved, and field experiments do not always show similar results as controlled laboratory or greenhouse studies.

Corn yield is more responsive to fungicide applications when hybrids have fair to poor resistance to disease, yields are below 9.1 Mg ha^{-1}, and disease severity is > 5% (Paul et al., 2011). The effects of fungicides on wheat yield are closely correlated with extending the duration of green leaf area by controlling disease (Gooding et al., 2000). Application of strobilurin fungicides have been shown to significantly increase yield compared to non-treated controls in multiple studies (Bertelson et al., 2001; Diaz-Espejo et al., 2012; Gooding et al., 2000; Ruske et al., 2003b), but these applications have had no effect or inconsistent effects due to hybrid selection and the timing of application in other studies (Blandino and Reyneri, 2009; Bradley and Ames, 2009; Inagaki et al., 2009). A meta-analysis, a "quantitative synthesis of research findings from multiple individual trials", on yield response of corn to foliar fungicides showed that in general, yield did increase compared to non-treated control when averaged across all trials in the meta-analysis (Paul et al., 2011). Of these trials, approximately 70% included disease ratings, and of the trials with disease ratings, about half of them had < 5% disease severity. However, based on the current application costs and grain prices, the authors concluded

that the chance of not recovering the cost of application to be > 75% when disease severity assessed between R4 and R5 was < 5%, and the chance of losing money by applying fungicides was < 25% when disease was > 5%. These authors also concluded that even when disease levels are > 5%, a profitable response to fungicide application is unpredictable because so many factors are involved in yield response.

Application of strobilurin fungicides on wheat and soybeans has been shown to increase individual grain weight, contributing to an increase in yield (Gooding et al., 2000; Henry et al., 2011). Mean grain weight increased due to the application of strobilurin fungicides on diseased wheat, which contributed to an increase in yield (Gooding et al., 2000). Maximum grain water content in wheat determined by weekly assessments of grain moisture is correlated with the final grain weight, meaning that the final grain weight is higher in grains that obtained a higher maximum water content during the growing season compared to grains that obtained a lower maximum water content (Dimmock and Gooding, 2002). This suggests that increasing the duration of green leaves after the maximum grain water content has been reached has little effect on yield or grain weight (Dimmock and Gooding, 2002). In a separate study, strobilurin fungicides had no effect on grain weight of wheat or green leaf duration (Blandino and Reyneri, 2009).

Grain moisture was significantly higher in corn when strobilurin fungicides were applied, which was probably due to delayed senescence compared to diseased, non-treated controls (Bradley and Ames, 2009). Byamukama et al. (2013) conducted an experiment to assess the effects of pyraclostrobin applied at VT/R1 on grain-fill period extension and kernel dry matter accumulation in corn. The authors noted that

pyraclostrobin delayed senescence of the upper canopy even under low (<5%) disease severity on the earleaf at R4 (kernel dough stage). However, delaying senescence had no effect on the timing of physiological maturity, length of grain-filling period, grain yield, or grain moisture. Grain yield did not have a significant linear relationship with delayed canopy senescence.

1.5.8 Oxidative Stress Resistance

Oxidative stress and active oxygen species can have an effect on plant aging and senescence by inducing pigment degradation, altering membrane permeability, and breaking down proteins (Wu and Tiedemann, 2002). Ozone causes more damage to crops in industrialized countries than any other pollutant (Wu and Tiedemann, 2002). Spring barley grown under greenhouse conditions had visibly less ozone injury in a fumigation chamber when treated with azoxystrobin compared to the non-treated control (Wu and Tiedemann, 2002). The authors found that treated plants showed increased levels of superoxide dismutase (SOD) in leaves, which works to suppress the damaging accumulation of oxides in leaf tissue (Wu and Tiedemann, 2002). Spring wheat (*Triticum aestivum* L.) treated with fungicides maintained higher levels of leaf protein content compared to non-treated control plants when subjected to superoxides (Wu and Tiedemann, 2001). Senescence was found to be delayed after exposure to superoxides due to delayed protein degradation and less ion leakage caused by application of azoxystrobin (Wu and Tiedemann, 2001).

1.5.9 Crop Canopy Temperature Responses

A variety of biotic and abiotic factors can influence the temperature of plant canopies by disrupting the transpiration stream, including water stress, insects and disease, salinity, and nutrient stress (Jackson et al., 1986). The canopy temperature of sugar beets (*Beta vulgaris* L.) and cotton (*Gossypium* sp.) infected with soil-borne fungal pathogens was significantly higher than the non-infected control plants (Pinter et al., 1979). Plants that were slightly diseased (< 10% of root area affected) did not show a change in temperature, but when fungal infection was > 10%, the temperature increased by 2.6 to 3.6ºC in sugar beets and 3.3 to 5.6ºC in cotton (Pinter et al., 1979). Multiple studies have shown that canopy temperature increases under water deficit, so temperature could be used to determine irrigation scheduling (Clawson and Blad, 1982; Gardner et al., 1981; González-Dugo et al., 2006). A 1ºC increase in temperature compared to a well-watered plot or temperature variation of 0.8ºC or greater within a plot is an effective tool to indicate a need for irrigation (Clawson and Blad, 1982). Gardner et al. (1981) also concluded that higher midday standard deviations (> 0.3ºC) in plant canopy temperatures indicate water stress in corn.

Very little research has been reported that documents the effects of fungicides on canopy temperature. Some studies indicated that the application of strobilurin fungicides caused stomatal conductance to decrease causing a decrease in transpiration (Grossman et al., 1998; Nason et al., 2007). From these findings, one could speculate that the canopy temperatures may increase slightly after the application of strobilurin fungicides due to reduced transpiration, but neither study included canopy temperature measurements. The temperature of bread wheat genotypes increased significantly in a controlled environment

one day after application of pyraclostrobin, but it ultimately remained at a lower temperature four days after the application than the non-treated control (Inagaki et al., 2009). The authors added that potted soil of the treated plants had higher water content than the potted soil of the untreated control, which may have caused the differences in leaf temperature. It was concluded that pyraclostrobin depressed root water uptake, resulting in slowed soil drying. The authors could not detect any temperature differences between treated and non-treated wheat plants in a field experiment at flowering when an application was made at the boot growth stage (Feekes 10) (Inagaki et al., 2009).

1.5.10 Resistance

The highly specific mode of action for QoI fungicides is a major strength, but also has been proven to be a weakness (Fernández-Ortuño et al., 2008). FRAC (2013) classifies QoI fungicides as having a high risk of developing resistance. Resistance of fungi to the QoI mode of action primarily arises from mutations in the mitochondrial cytochrome *b* gene resulting in peptide sequence changes that prevent the fungicide from binding at the target site (Fernández-Ortuño et al., 2008). Some fungi have also developed resistance through creating an alternative route of respiration in which the electrons are transferred directly from the ubiquinol pool to oxygen without passing through the cytochrome bc_1 target site (Köller et al., 2001). Fortunately, the alternative route of respiration seems to only counteract QoI effectiveness *in vitro* (partial organism), not *in vivo* (whole, living organsim) (Fernández-Ortuño et al., 2008; Köller et al., 2001).

Fifty-six pathogens have already developed resistance to QoI fungicides (FRAC, 2013). At this point in time, *Ustilago maydis* (smut) is the only corn pathogen resistant to

QoI fungicides (FRAC, 2013). Seven pathogens that affect wheat, including *Septoria tritici* (leaf spot), have developed resistance to QoI fungicides (FRAC, 2013). One soybean pathogen, *Cercospora sojina* (frogeye leaf spot), developed resistance to QoI fungicides after only 4 years of use (Mueller et al., 2013). The previous examples show the importance in taking the steps necessary to prevent more QoI-resistant fungal strains from developing in the near future.

Fungicides that have a single-site mode of action tend to be at a higher risk for pathogens to become resistant than fungicides with multi-site activity (Mueller et al., 2013). Fungal pathogens that reproduce sexually and have a polycyclic life cycle are more likely to develop resistance (Mueller et al., 2013). In order to slow down or prevent resistance of target pathogens to QoI fungicides, it is very important to follow the instructions on the product label (Brent and Hollomon, 2007). The product label for a fungicide indicates the maximum number of applications and rate that can be applied to a crop each growing season. Exceeding these recommendations increases the potential for fungi to develop resistance, as do unnecessary applications of fungicides within this mode of action (Brent and Hollomon, 2007). Fungicides should only be applied when the risk is high for disease development because excess applications increase the chances of selecting for fungicide-resistant pathogen strains (Mueller et al., 2013). Programs to monitor the baseline level, amount of fungicide needed for controlling fungal pathogens, may be implemented to monitor the sensitivity of a fungal pathogen to specific fungicides (Mueller et al., 2013). Mixing fungicides of different FRAC groupings or applying pre-mixed fungicides is another way to slow the development of resistant pathogens (Mueller et al., 2013).

1.6 Foliar Applications of Nutrients

1.6.1 Nitrogen Availability and Uptake

Nitrogen (N) is needed in greater quantities than all other essential mineral nutrients in corn production systems. Depending on environmental conditions, N can make up approximately 2% of total plant dry matter (Miller and Cramer, 2004). Plants acquire N via the roots in the form of nitrate (NO_3^-) or ammonium (NH_4^+) and are key components in the production of proteins, nucleic acids, chlorophyll, co-enzymes, and phytohormones (Marschner, 2012). Minor inputs of N are provided to plants via the seed, atmospheric deposition, irrigation waters, crop residues, and animal manures (Smil, 1999). Legumes are able to utilize atmospheric N_2 with the help of symbiotic N_2 fixing bacteria in the soil to provide substantial amounts of N for plant growth and development (Smil, 1999). Most cropping systems without legumes, however, require large amounts of synthetic inorganic N fertilizers to meet plant demands (Smil, 1999). N losses occur due to denitrification, volatilization, leaching, soil erosion, and senescing plant parts (Smil, 1999). Application of large amounts of N fertilizer needed to meet plant demands and low N use efficiency increase the risk of surface water and groundwater contamination, which is of great concern in trying to maximize yield in modern cropping systems while minimizing impacts on the environment (Miller and Cramer, 2004).

Availability of nutrients in the soil are dependent on the three components of nutrient uptake: root interception, mass flow, and diffusion (Marschner, 2012). Nitrogen is primarily taken up by the roots through the processes of mass flow and diffusion (Miller and Cramer, 2004). Plant available N in the soil is influenced by many factors including soil texture, pH, soil moisture, temperature, and microbial activity (Marschner,

2012). Low soil moisture restricts transport of nutrients to the roots by decreasing diffusion rates in the soil and decreasing transpiration rates, thus reducing the rate of mass flow (Hu et al., 2008). Foliar applications of essential nutrients, especially N, have been suggested as a way to correct nutrient deficiencies, increase grain yield under nutrient-limiting soil conditions, and reduce the potential of environmental concerns by being more targeted on the plant (Giskin and Efron, 1986; Harder et al., 1982; Hu et al., 2008; Marschner, 2012).

1.6.2 Foliar Fertilization with Nitrogen

Multiple factors affect the absorption and plant response of foliar sprays including chemical properties of the spray formulation, environmental factors, and physiological status of the plant (Fernández et al., 2013). The uptake of a nutrient through foliage consists of foliar adsorption, penetration of the cuticle or stomata, uptake and absorption into metabolically active leaf cells, and then translocation to areas of the plant where it is needed (Fernández et al., 2013). Under normal conditions, active transport and consequently, energy are required to take up ions against a gradient (Fageria et al., 2009). Absorption is most efficient when light quality and intensity are high, stomata are open, temperatures are low, a plant is cool and turgid, and wind levels are low (Fageria et al, 2009). Urea is absorbed at a more rapid rate and in greater quantities than other inorganic forms of N (Wójcik, 2004). Once the leaf absorbs N, it must still be metabolized before it can be utilized for plant essential processes (Wójcik, 2004), which is similar to the process of N uptake by the roots (Marschner, 2012).

Although foliar application of nutrients seems like a convenient alternative to the typical standard of soil fertilization practices, Marschner (2012) listed several potential problems with foliar applications of nutrients: limited spreading on leaf surface, spray run-off, rain before nutrients are absorbed, low leaf penetration rates, rapid drying of spray solutions, limited distribution throughout the plant, phytotoxicity concerns, leaf damage, and potential nutrient imbalances. Gamble and Emino (1987) conducted a study on leaf burn in corn induced by foliar-applied N. After applying one drop of urea at 120 g N L^{-1}, the authors observed three fates: rapid drying of the urea leaving salt on the leaf surface with no injury, absorption of the urea without any trace of residue along with desiccation of the epidermal cells, or formation of a gel-like deposit along with collapse and desiccation of the epidermis resulting in lesion formation. Applying the same rate of N at higher concentrations results in a greater amount of leaf burn and decreased effectiveness (Foy et al., 1953). Only about 30% of foliar-applied N is absorbed into corn plants (Below et al., 1985). Plant nutritional requirements cannot be met by foliar applied nutrients alone, and deficiencies may appear under unfavorable environmental conditions for adsorption (Fageria et al., 2009).

Foliar-applied nutrients in corn production have had mixed effects on corn production. Several studies have reported little to no effect on grain yield in response to foliar nutrient sprays, and some have reported a negative yield response to foliar-applied N (Below et al., 1984a; Dobbs et al., 2003; Foy et al., 1953; Harder et al., 1982; Ippersiel et al., 1989). Some studies have shown an increase in grain N concentrations when foliar sprays were applied shortly after anthesis (Below et al., 1984a; Harder et al., 1982; Ippersiel et al., 1989). Giskin and Efron (1986) noted an increase in grain yields and

silage yields after applying a foliar solution of N, P, K, and S at the four to five leaf stage. The authors reported a significant increase in seedling N and P concentrations at a critical growth stage shortly after application, which most likely contributed to the positive yield responses.

Harder et al. (1982) studied the effects of applying a solution of N, P, K, and S to corn at different times shortly after silking under different moisture regimes. There was no effect of foliar fertilizer on grain yield or yield components in the first year of the study. There was a significant reduction in weight kernel^{-1} contributing to reduced grain yield in the second year, which was most likely the result of foliar injury. The authors did, however, note a 10% increase in grain N concentration, and an increase in the overall quantity of N in the grain in response to the foliar spray solution. There was no interaction of foliar fertilization with moisture-stress treatments. A similar study conducted by Below et al. (1984a, 1984b) yielded similar results. The authors saw no response on grain yield or total plant dry weight, but they did see an increase in grain N concentration. Increases in grain protein indicate that ears are an effective sink for excess N at the post-anthesis stage of development. An increase in stalk lodging in response to the foliar sprays of N, P, K, and S was noted in this study. The liquid fertilizer CoRoN® (25-0-0; Helena Chemical Company, Collierville, TN) applied at 9.35 or 18.70 L ha^{-1} at V12 caused about five percent leaf damage and had no effect on grain yield (Dobbs et al., 2003). This particular foliar spray is made up of 18.8% urea and 6.2% methylene urea and methylene diurea. The failure to increase grain yields may be in part due to foliar-derived N being incorporated into a readily remobilized pool of N (Below et al., 1985).

1.6.3 Boron

Boron (B) is the most widespread micronutrient deficiency in the United States for a number of crops such as alfalfa (*Medicago saliva* L.), fruit trees, clover (*Trifolium pratense* L.), etc. in 41 states (Berger, 1962). However, incidences of B deficiency in corn are sparse (Sparr, 1970). Boron is a micronutrient that is essential for plant growth and development. Little work has been done with foliar applications of B on corn; however, B is often not a limiting nutrient in Midwest corn production. Corn typically only takes up 0.18 kg B ha^{-1} and is reported to have a very low sensitivity to B deficiency (Martens and Westermann, 1991). Exact roles of B are not completely understood, but B is thought to be involved in: cell wall structure, plasma membrane processes, metabolism, and utilization of absorbed light energy (Marschner, 2012). Plant uptake is closely related to external concentrations, so caution must be taken when applying B to crops to avoid toxicity (Marschner, 2012). Boron toxicity has shown to significantly reduce overall plant biomass (Ben-Gal, 2007). The availability of B in the soil depends on pH, soil texture, organic matter, clay mineralogy, and especially soil moisture (Marschner, 2012). Dry conditions reduce transpiration rates, so the primary means of B transport, mass flow, is limited (Marschner, 2012). Boron is easily leached from the soil profile and can be limiting in areas receiving high amounts of rainfall (Marschner, 2012). The mobility of B inside the plant is very low, so a constant supply is necessary to reach all plant parts (Berger et al., 1957). The highest B concentrations are typically found in the tassel, followed by the upper leaves, and then lower leaves, and concentrations are greater in leaf margins than leaf midribs (Touchton and Boswell, 1975).

Lordkaew et al. (2011) conducted a study to determine the effects on corn growth and development in B deficient soils. The authors observed that B deficiency did not become obvious until reproductive growth stages. Silks and tassels were much smaller on deficient plants than those with normal levels of B. In some cases, the primary ear behaved as a tassel. Most anthers lacked pollen or had less pollen than control plants, and the authors found that B was essential for pollen germination. It was also noted that silks were non-receptive under B deficiency. Berger et al. (1957) found that B-deficient plants often did not produce ears or produced barren ears.

1.6.4 Boron Fertilization

Studies have shown mixed results on which method of application is most effective at correcting B deficiencies. One study looking at the effectiveness of different methods for correcting B deficiency showed that row application of B provided greater uptake than both foliar sprays and broadcast applications (Peterson and MacGregor, 1966). Other studies have shown that foliar applications provide the plant with more B than soil applications (Ben-Gal, 2007; Touchton and Boswell, 1975). Even though earleaf concentrations were higher after foliar treatments of B, there was no yield response in soils with sufficient B (Touchton and Boswell, 1975). Berger et al. (1957) observed a yield increase in response to B applications in 6 of 54 fields. If B deficiency develops, mid-season foliar applications have proven to be effective in correcting these problems (Ben-Gal, 2007; Touchton and Boswell, 1975).

1.7 Crop Canopy Reflectance

1.7.1 Active Optical Reflectance Sensors

Optical properties of plant canopies may be used to monitor crop status for making site-specific crop management decisions (Gunzenhauser and Shanahan, 2013; Hatfield and Pinter, 1993; Hatfield et al., 2008). Light may be absorbed, reflected, or transmitted when it reaches a plant surface (Hatfield and Pinter, 1993). Remote sensing tools, both passive and active, measure the amount of light that is reflected from the crop canopy (Gunzenhauser and Shanahan, 2013). Passive sensors measure the amount of light reflected by the sun, and active sensors emit their own light (Gunzenhauser and Shanahan, 2013). Light emitted by active optical sensors is modulated, which allows the sensors to differentiate between natural light and emitted light (Holland Scientific, 2008). Spectrally sensitive photosensors detect the amount of emitted light reflected from the plant canopy (Holland Scientific, 2008). By emitting their own light source, active sensors have the ability to operate under any type of lighting condition (Kim et al., 2010).

1.7.2 Optical Properties of Pigments

Remote sensing has proven to be a valuable technique to monitor crop status in multiple studies (Gitelson et al., 2006; Hatfield et al., 2008; Solari et al., 2008). Plants capture light energy from photosynthetically active radiation (PAR) which ranges from 400 to 700 nm for use in photosynthesis (Blankenship, 2002). Most of the light for photosynthesis is absorbed by antenna pigments, consisting of chlorophylls *a* and *b* and carotenoids (Blankenship, 2002). Therefore, the amount of PAR absorbed by plants is a function of the concentrations of antenna pigments (Richardson et al., 2002). Leaves

with low levels of chlorophylls *a* and *b* typically have high reflectance in PAR and low reflectance in the near infrared range (NIR; 700-1000 nm) (Daughtry et al., 2000; Filella and Penuelas, 1994). Chlorophyll content tends to be highest at tasseling (VT) and starts to decline throughout the reproductive stages (Ciganda et al., 2008). During vegetative growth stages, differences in reflectance are largely related to the amount of biomass accumulation (Viña et al., 2004).

Chlorophylls absorb energy very strongly centered in the blue (≈450 nm) and the red (≈670 nm) region of PAR, and carotenoids absorb wavelengths centered around the blue region only (Hatfield et al., 2008; Sims and Gamon, 2002). Low levels of pigments are sufficient to saturate absorption in the blue and red regions because the depth of light penetration into the leaf is low (Daughtry et al., 2000; Hatfield et al., 2008). Canopy reflectance in the green (≈550 nm) and red-edge (≈715 nm) regions are more sensitive to changes in pigment concentration when levels of pigments are medium to high (Daughtry et al., 2000; Hatfield et al., 2008; Schlemmer et al., 2013). Increases in either chlorophyll content or leaf area index (LAI) cause the position of the red-edge to shift toward longer wavelengths (Daughtry et al., 2000; Filella and Penuelas, 1994). Reflectance in the NIR region is very high, and it is strongly influenced by the internal leaf structure (Gausman et al., 1984). Near-infrared reflectance tends to increase as the mesophyll structure develops, but reflectance is fairly resistant to change with minor external factors and pigment concentration once the mesophyll is developed (Gates, 1970; Gitelson et al., 1996). Canopy reflectance is strongly affected by the concentration of antenna pigments, leaf area index (LAI), and background reflectance (Daughtry et al., 2000).

1.7.3 Vegetative Indices

Multiple studies have found strong correlations between crop canopy reflectance and leaf chlorophyll content (Ciganda et al., 2008; Daughtry et al., 2000; Fillella and Penuelas, 1994; Houborg et al., 2009; Schlemmer et al., 2013). A number of studies have also found strong correlations between canopy reflectance and LAI and biomass (Filella and Penuelas, 1994; Gitelson et al., 2003; Houborg et al., 2009; Viña et al., 2004). Leaf chlorophyll content is highly related to leaf N content, so multiple studies have been successful in estimating N content remotely (Schlemmer et al., 2013; Shiratsuchi et al., 2011; Solari et al., 2008). Vegetative indices have been developed in an attempt to minimize the effects of changing soil backgrounds and illumination intensities (Hatfield and Pinter, 1993). Two common indices have been used to estimate chlorophyll content and LAI:

Chlorophyll Index (CI; Ciganda et al., 2008; Schlemmer et al., 2013):

$$CI = (NIR/PAR)-1$$

Normalized Difference Vegetative Index (NDVI; Gitelson et al., 1996; Schlemmer et al., 2013):

$$NDVI = (NIR-PAR)/(NIR+PAR)$$

1.7.4 Optical Properties Under Environmental Stress

Plants tend to exhibit similar symptoms under a variety of stresses, two of which include a reduction in LAI (or stunted growth) and a decrease in chlorophyll content (Baret et al., 2007; Hatfield et al, 2008). A variety of stresses including plant competition, herbicide damage, pathogens, ozone damage, and drought conditions all increase

reflectance near the green and red region of the spectrum in multiple species (Carter, 1993). In a study on corn, canopy reflectance increased in the chlorophyll absorption region 1.5 days after inoculation with *Helminthosporium maydis* (Southern corn leaf blight; Safir et al., 1972). A study on barley inoculated with *Erysiphe graminis* f. sp. *hordei* (Powdery mildew) demonstrated increased reflectance in PAR due to the degradation of chlorophyll and decreased reflectance in the NIR region caused by deterioration of cell walls (Lorenzen and Jensen, 1989). Bravo et al. found similar results in a study on wheat infected with *Puccinia striiformis* f. sp. *tritici* (Yellow rust; 2003). However, reflectance in the NIR region did not change in a study on rice (*Oryza sativa* L.) infected with *Magnaporthe grisea* Barr. (Rice panicle blast; Kobayashi et al., 2001). Canopy reflectance alterations due to disease may vary with fungal pathogens depending on the biology of each species (Malthus and Madeira, 1993).

1.7.5 Chlorophyll Meters

The SPAD chlorophyll meter (Konica Minolta Optics, Inc., Tokyo, Japan) measures the amount of light that is transmitted through a leaf (Markwell et al., 1995). SPAD chlorophyll meters emit red (650 nm) and infrared (940 nm) light in sequence through a leaf and detect the transmittance with a photodiode receptor (Markwell et al., 1995). A value (SPAD unit) is output based on the ratio of light transmittance, and this value is related to the chlorophyll content in that leaf (Markwell et al., 1995). Chlorophyll meters have proven to be a valuable tool for estimating leaf chlorophyll content over a variety of conditions (Richardson et al., 2002; Schepers et al., 1992;

Uddling et al., 2007). Leaf N content and chlorophyll meter values are highly correlated, allowing one to estimate N content in a noninvasive manner (Chapman and Barreto, 1997; Schepers et al., 1992).

1.8 Summary

Foliar fungicide use has increased substantially in recent years as a result of multiple factors: development of new fungicide products labeled for corn, increased grain market prices, increased incidence and severity of foliar diseases as a result of increased no-till and continuous corn cropping practices, and fungicide manufacturer claims. The "Plant Health™" marketing campaign is very appealing to corn producers looking to optimize yields. Some of the marketing claims include more vigorous plant growth, improved stalk strength, N utilization, tolerance to hail damage, tolerance to environmental stress, better harvestability, and ultimately, increased grain yield even in the absence of disease. Many fungicide manufacturers are also promoting early season (V4-V8) applications of foliar fungicides. Controlled greenhouse studies have shown fungicide application to improve N utilization (Köhle et al., 2002), reduced stress and delayed senescence (Grossmann and Retzlaff, 1997), reduced plant defense mechanisms (Smedegaard-Petersen and Tolstrup, 1985), and oxidative stress resistance (Wu and Tiedemann, 2002). However, studies conducted in the field under natural growing conditions do not always show a yield benefit from the application of foliar fungicides (Bradley and Ames, 2009; Byamukama et al., 2013; Paul et al., 2011).

Foliar applications of essential nutrients have increased in popularity in recent years with the development of new products. Many fertilizer manufacturers claim that

slow-release forms of nutrients are more efficient than soil-applied nutrients. The availability of new products, manufacturer claims, high grain prices, and ease of application has contributed to this recent interest in foliar fertilizers. Previous studies have not shown foliar-applied N to be a beneficial practice (Below et al., 1984; Harder et al., 1982). However, foliar sprays have proven to be an effective means of correcting micronutrient deficiencies mid-season (Ben-Gal, 2007; Touchton and Boswell, 1975).

The objectives of this study were to 1) evaluate the effects of V5 applications, R1 applications, and a combination of V5 and R1 applications of commonly utilized foliar fungicides on overall plant health and grain yield and 2) evaluate the effects of a V5 foliar N fertilizer application on overall plant health and grain yield.

CHAPTER 2. EFFECT OF FUNGICIDES AND A FOLIAR FERTILIZER ON OVERALL PLANT HEALTH AND GRAIN YIELD IN CORN

2.1 Introduction

Corn (*Zea mays* L.) is the most widely grown grain crop in the United States with about 38.6 million ha planted in 2013 (USDA-NASS, 2014). Approximately 50% of the total crop acreage in Indiana is planted in corn (USDA-NASS, 2014). Traditionally, fungicides have only been used on seed or specialty corn crops; however, fungicide use on dent corn has increased tremendously since 2007, with an estimate of over 4 million ha of corn now being treated (Wise and Mueller, 2011). The recent increase in fungicide use on corn is the result of multiple factors: availability of new fungicides, increased grain market price of corn, manufacturer claims of yield increases due to fungicide applications, increased incidence and severity of foliar diseases as a result of increased no-till and reduced tillage cropping practices (Wise and Mueller, 2011). According to Jardine and Ciampitti (2013) fungicide manufacturers currently promote early season (V4 to V8; Abendroth et al., 2011) fungicide applications in addition to the traditional application timing of VT to R1. Manufacturers also promote the use of fungicides to reduce stress and improve overall plant health through physiological responses even in the absence of disease. As a result, many producers now make "insurance" applications of foliar fungicides. Prophylactic fungicide use is of great concern with these newer single-site mode of action fungicides because the fungicides are at a higher risk for

becoming ineffective due to the development of resistant pathogens, compared to some of the older multi-site mode of action fungicides (FRAC, 2013).

A supplemental label (EPA Reg. No. 7969-186) claims that application of the strobilurin fungicide pyraclostrobin (Headline®; BASF Corporation, Research Triangle Park, NC) provides improved plant health and tolerance to environmental stresses, such as drought, heat, cold, and ozone damage (BASF, 2008). This supplemental label also states that application can provide "improved utilization of N…., improved stalk strength and better harvestability, induced tolerance to stalk diseases, better tolerance to hail, and more uniform seed size" (BASF, 2008).

Foliar pathogens cause lesions or blighting of photosynthetic plant tissue, making those areas nonfunctional, which can reduce overall grain yield depending on severity (Ward et al., 1999). Reducing the photosynthetic leaf area in corn may also indirectly lead to stalk rot and lodging by increasing the amount of carbohydrates transferred from the stalk to the developing ear during grain filling (Dodd, 1980). Transpiration rates, permeability of leaf cells, and respiration rates also increase in diseased plants (Agrios, 2005). The percentage of overall yield loss is largely affected by the timing of disease development and location in the plant canopy (Stuckey et al., 1993). Yield loss is typically greater when onset of disease in the upper plant canopy occurs around R1 and continues at least 2-3 weeks after pollination (Adee et al., 2005; Levy and Pataky, 1992).

Common foliar fungal diseases in the Midwest include gray leaf spot (GLS) caused by *Cercospora zeae-maydis*, northern corn leaf blight (NCLB) caused by *Exserohilum turcicum*, and anthracnose leaf blight (ALB) caused by *Colletotrichum graminicola* (Bergstrom and Nicholson, 1999; Mueller et al., 2013; Perkins, 1987). All

of these pathogens overwinter within infected corn residue on the soil surface (Bergstrom and Nicholson, 1999; Levy and Pataky, 1992; Ward et al., 1999). For this reason, crop rotation and tillage are very effective at reducing levels of primary inoculum (Ward et al., 1999). Selecting resistant hybrids is one of the simplest and most effective methods of disease control (Perkins, 1987; Ward et al., 1999). Fungicides can be very effective at controlling fungal diseases depending on the timing of application; however, the inability to predict disease severity or the potential yield impact of the disease makes the decision to apply fungicides difficult (Ward et al., 1997; Munkvold et al., 2001). The probability of preventing yield loss with a fungicide application is greater when disease severity is high, hybrid resistance is low, corn residue levels are high, and weather conditions are conducive for disease (Mueller et al., 2013).

Fungicides evaluated in this study are classified as strobilurin fungicides, and they are part of the Quinone outside Inhibitors or QoI – fungicides (FRAC Code 11). These fungicides act by binding to the quinol oxidation site of the cytochrome bc1 complex, inhibiting the transfer of electrons (Mueller et al., 2013). This inhibits mitochondrial respiration in fungi, which halts energy production and ultimately leads to death (Mueller et al., 2013). Some of the fungicides in this study contain Demethylation Inhibitors or DMI – fungicides (FRAC Code 3) in addition to the QoI active ingredient. Demethylation inhibitor fungicides inhibit the C14-demethylase enzyme in fungi which is essential for membrane structure and function (Mueller et al., 2013). Both groups of fungicides are typically locally systemic in plants and provide preventative and some early infection protection against foliar fungal pathogens (Mueller et al., 2013).

Strobilurin fungicides have a "greening effect", whereby treated plants maintain green leaf area longer in the growing season than non-treated plants (Bartlett et al., 2002; Bertelson et al., 2001; Byamukama et al., 2013; Ruske et al., 2003b; Grossmann and Retzlaff, 1997). In wheat (*Triticum aestivum* L.), fungicide application increased green leaf area duration and grain N due to delayed senescence of the flag leaf resulting from control of disease (Ruske et al., 2003b). Applying strobilurin fungicides indirectly reduced the production of ethylene; consequently reducing the loss of chlorophyll and delaying plant senescence (Grossmann and Retzlaff, 1997). Fungicides inhibited senescence-promoting activity of saprophytes and reduced plant defense mechanisms and energy expenditure, which ultimately delayed leaf senescence in winter wheat (Bertelson et al., 2001). In corn, fungicides extended green leaf duration, but they had no effect on grain yield, grain moisture, or length of grain-fill period (Byamukama et al., 2013).

Other physiological effects on plants have been observed in plants after receiving an application of strobilurin fungicides when grown in controlled environments; however, these changes were often not detectable in field experiments. Strobilurin fungicides have been reported to reduce abscisic acid (ABA) levels in wheat plants grown in growth chambers, which reduced water consumption by 8% due to reduced transpiration rates compared to the control (Grossmann et al., 1998). In the field, no effects were observed on ABA content and stomatal conductance on grapevines (*Vitis vinifera* L.) after a strobilurin application (Diaz-Espejo et al., 2012). Strobilurin fungicides have been reported to improve water use efficiency (WUE) of wheat, soybean (*Glycine max* Merr.), and barley (*Hordeum vulgare* L.) in greenhouse conditions under well-watered conditions,

but they decreased WUE in water-deficit conditions (Nason et al., 2007). One study found that azoxystrobin was effective at reducing ozone damage in spring barley grown in greenhouse conditions (Wu and Tiedemann, 2002).

Yield response of plants to fungicide applications is affected by multiple factors, some of which include disease pressure, hybrid susceptibility, environmental conditions, and timing of applications (Henry et al., 2011; Paul et al., 2011; Ruske et al., 2003b). Strobilurin fungicides were effective in increasing grain yield compared to the control in several studies (Bertelson et al., 2001; Diaz-Espejo et al., 2012; Gooding et al., 2000; Ruske et al., 2003b), however, these fungicides had no effect or inconsistent effects in other studies due to hybrid selection and the timing of application (Blandino and Reyneri, 2009; Bradley and Ames, 2009; Inagaki et al., 2009). A meta-analysis, a "quantitative synthesis of research findings from multiple individual trials", on yield response of corn to foliar fungicides showed that in general, yield did increase compared to non-treated control when averaged across all trials in the meta-analysis (Paul et al., 2011). In general, corn yield was more responsive to fungicide applications when hybrid disease resistance was rated as fair to poor resistance to disease, grain yield without fungicides was <9.1 Mg ha^{-1}, and disease severity was >5% on untreated plants (Paul et al., 2011).

Foliar fertilization has also increased in popularity in recent years due to the increase in corn prices and manufacturer claims. Fertilizer manufacturers have begun to promote slow-release foliar fertilizers that are purported to be more efficient than traditional soil-applied N fertilizers (Sawyer, 2009).

In this study, we assessed the effects of CoRoN®, a foliar fertilizer with an analysis of 25-0-0 plus 0.5% B, when applied at the V5 growth stage. This formulation

of CoRoN contains 18.8% urea N, 6.2% slowly available N from methylene diurea and methylene urea, and 0.5% boron. Nitrogen is needed in greater quantities than all other essential mineral nutrients in corn production systems. Depending on environmental conditions, N can make up approximately 2% of total plant dry matter (Miller and Cramer, 2004). Plants acquire N via the roots in the form of nitrate (NO_3^-) or ammonium (NH_4^+) and produce proteins, nucleic acids, chlorophyll, co-enzymes, and phytohormones (Marschner, 2012). Large amounts of synthetic inorganic N fertilizers are required to meet corn plant demands (Smil, 1999). Available N in the soil is largely dependent on soil texture, soil pH, soil moisture, temperature, and microbial activity (Marschner, 2012). Low soil moisture restricts transport of nutrients to the roots by decreasing diffusion rates in the soil and decreasing transpiration rates, thus reducing the rate of mass flow (Hu et al., 2008). Foliar applications of essential nutrients, especially N, have been suggested as a way to correct nutrient deficiencies, increase grain yield under nutrient-limiting soil conditions, and reduce the potential of environmental concerns by being more targeted on the plant (Giskin and Efron, 1986; Harder et al., 1982; Hu et al., 2008; Marschner, 2012).

Just like soil-applied nutrients, multiple factors affect the absorption and plant response of foliar-applied nutrients: chemical properties of the nutrient solution, environmental factors, and the physiological status of the plant (Fernández et al., 2013). Nutritional requirements for macronutrients of plants cannot be met by foliar-applied nutrients alone because a limited amount of the applied nutrients are actually absorbed and applying the required rate often results in phytotoxicity and leaf damage (Below et al., 1985; Fageria et al., 2009; Foy et al., 1953; Gamble and Emino, 1987). Several studies on corn have reported an increase in grain N concentrations when foliar N was applied

shortly after anthesis (Below et al., 1984a; Harder et al., 1982; Ippersiel et al., 1989); however, there is typically little to no effect on overall grain yield in response to foliar-applied nutrients (Below et al., 1984a; Dobbs et al., 2003; Foy et al., 1953; Harder et al., 1982; Ippersiel et al., 1989).

Boron (B) is a micronutrient that is essential for plant growth and development. Corn typically takes up about 0.18 kg B ha^{-1} and is reported to have low sensitivity to B deficiency (Martens and Westermann, 1991). Specific roles of B are not completely understood, but B is thought to be involved in cell wall structure, plasma membrane processes, metabolism, and utilization of absorbed light energy (Marschner, 2012). The amount of plant available B in the soil largely depends on soil pH, soil texture, organic matter, clay mineralogy, and especially soil moisture (Marschner, 2012). Deficiency symptoms specific to corn include under-sized silks and tassels, low levels of pollen, non-receptive silks, and barren ears (Berger et al., 1957; Lordkaew et al., 2011). Foliar applications of B can effectively correct mid-season B deficiencies in corn (Ben-Gal, 2007; Touchton and Boswell, 1975).

Optical reflectance properties of plant canopies can be used to monitor crop status throughout the growing season (Gunzenhauser and Shanahan, 2013). Plants capture photosynthetically active radiation (PAR), 400 to 700 nm, for use in photosynthesis (Blankenship, 2002). Most of the light for photosynthesis is absorbed by chlorophylls *a* and *b* and carotenoids, so the amount of PAR absorbed by plants is a function of the concentration of these pigments (Blankenship, 2002; Richardson et al., 2002). Chlorophylls absorb energy very strongly centered in the blue (≈450 nm) and red (≈670 nm) regions of PAR (Hatfield et al., 2008). Canopy reflectance in the green (≈550 nm)

and red-edge (≈715 nm) regions are more sensitive to changes in pigment concentration (Hatfield et al., 2008). Reflectance is very high in the near infrared range (NIR; 700-1000 nm), and it is strongly influenced by internal leaf structure (Gausman et al., 1984). Crop canopy reflectance is largely influenced by the concentration of pigments and leaf area index (LAI) (Daughtry et al., 2000).

Multiple studies have found strong correlations between crop canopy reflectance and leaf chlorophyll content (Ciganda et al., 2008; Daughtry et al., 2000; Fillella and Penuelas, 1994; Houborg et al., 2009 Schlemmer et al., 2013). Leaf chlorophyll content and leaf N content was highly correlated ($R^2 = 0.73$) throughout the growing season in corn (Schlemmer et al., 2013). A number of studies have also found strong correlations between canopy reflectance and LAI and biomass (Filella and Penuelas, 1994; Gitelson et al., 2003; Houborg et al., 2009; Viña et al., 2004). Vegetative indices have been developed in an attempt to minimize the effects of changing soil backgrounds and illumination intensities (Hatfield and Pinter, 1993). Two common indices have been used to estimate chlorophyll content and LAI:

Chlorophyll Index (CI; Ciganda et al., 2008; Schlemmer et al., 2013):

$$CI = (NIR/PAR)-1$$

Normalized Difference Vegetative Index (NDVI; Gitelson et al., 1996; Schlemmer et al., 2013):

$$NDVI = (NIR-PAR)/(NIR+PAR)$$

Plants exhibit similar symptoms under a variety of stresses, two of which include a reduction in LAI (or stunted growth) and a decrease in chlorophyll content (Baret et al., 2007; Hatfield et al., 2008). A variety of stresses including plant competition, herbicide

damage, pathogens, ozone damage, and drought conditions all increase canopy reflectance near the green and red regions of PAR (Carter, 1993). A variety of plant species have exhibited increased reflectance in PAR in response to disease (Bravo et al., 2003; Lorenzen and Jensen, 1989; Safir et al., 1972).

The SPAD chlorophyll meter (Konica Minolta Optics, Inc., Tokyo, Japan) is often used to estimate leaf chlorophyll content over a variety of conditions (Richardson et al., 2002; Schepers et al., 1992; Uddling et al., 2007). SPAD chlorophyll meters operate by emitting red (650 nm) and infrared (940nm) light in sequence through a leaf and transmittance is quantified with a photodiode receptor (Markwell et al., 1995). It then outputs a value (SPAD unit) based on the ratio of light transmittance, and this value is related to leaf chlorophyll content (Markwell et al., 1995).

The objectives of this study were to 1) evaluate the effects of V5 applications, R1 applications, and a combination of V5 and R1 applications of several commonly utilized foliar fungicides on overall plant health and grain yield and 2) evaluate the effects of a V5 foliar N fertilizer application on overall plant health and grain yield.

2.2 Materials and Methods

Field experiments were conducted in 2012 and 2013 at the Purdue Agricultural Center for Research and Education (ACRE; 40.2909º, -87.0028º, elevation 215 m above sea level) near West Lafayette, IN; the Pinney Purdue Agricultural Center (PPAC; 41.2704º, -86.5628º, elevation 224 m above sea level) near Wanatah, IN; and the Southeast Purdue Agricultural Center (SEPAC; 39.0231º, -85.3125º, elevation 243 m above sea level) near Butlerville, IN. Field experiments were conducted at two additional

locations in 2013: the Davis Purdue Agricultural Center (DPAC; 40.1454º, -85.0900º, elevation 296 m above sea level) near Farmland, IN and the Northeast Purdue Agricultural Center (NEPAC; 41.0652˚, -85.2654˚, elevation 254 m above sea level) near Columbia City, IN. Complete soil descriptions are provided in Table 2-1.

Table 2-1. Description of soils and percentage of field by soil type for all locations used to examine the response of corn to V5 and R1 applications of fungicides and foliar fertilizer (V5 only) in 2012 and 2013.

Location	Percentage of field	Slope	Soil Series	Family
ACRE	44	0-2%	Chalmers	fine-silty, mixed, superactive, mesic Typic Endoaquolls
	56	0-1%	Raub-	fine-silty, mixed, superactive, mesic Aquic Argiudolls
			Brenton	fine-silty, mixed, supeactive, mesic Aquic Argiudolls
DPAC	59	0-1%	Blount	fine, illitic, mesic Aeric Epiaqualfs
	29	0-2%	Pewamo	fine, mixed, active, mesic Typic Argiaquolls
	10	1-4%	Glynwood	fine, illitic, mesic Aquic Hapludalfs
NEPAC	40	0-3%	Haskins	fine-loamy, mixed, active, mesic Aeric Eqiaqualfs
	30	2-6%	Rawson	fine-loamy, mixed, active, mesic Oxyaquic Hapludalfs
	18	3-6%	Glynwood	fine, illitic, mesic Aquic Hapludalfs
PPAC	100	0-2%	Sebewa	fine-loamy over sandy, mixed, superactive, mesic Typic Argiaquolls
SEPAC	41	0-2%	Avonburg	fine-silty, mixed, active, mesic Aeric Fragic Glossaqualfs
	25	2-6%	Nabb	fine-silty, mixed, active, mesic Aquic Fragiudalfs
	15	2-6%	Ryker-	fine-silty, mixed, active, mesic Typic Paleudalfs
			Muscatatuck	fine-silty, mixed, active, mesic Fragiaquic Paleudults
	12	0-1%	Cobbsfork	fine-silty, mixed, active, mesic Fragic Glossaqualfs

Corn was the previous crop at all locations. Tillage practices were no-till (SEPAC & NEPAC) and conventional tillage, consisting of either chisel or disc operations in the fall and one to two passes with a field cultivator in the spring (ACRE, DPAC, & PPAC). All locations were seeded at 81,500 plants ha^{-1} (except ACRE in 2012 was seeded at 79,100 seeds ha^{-1}), at a planting depth dictated by soil moisture conditions at the time of planting. Pioneer brand P0916XR (109 comparative relative maturity) was

planted at all locations except SEPAC, which was planted with LG 2625VT3 (114 day relative maturity). Pioneer brand P0916XR has a rating of 5 for both gray leaf spot (*Cercospora zeae-maydis*) and northern corn leaf blight (*Exserohilum turcicum*), and LG 2625VT3 has a rating of 8 for GLS and 7 for NCLB with 1 being poor and 9 being excellent resistance. Starter fertilizer was applied 5 cm beside and 5 cm below seed with rates varying among location (Table 2-2). A variety of herbicides were used throughout the growing season to control weeds. Plots were side-dressed with urea-ammonium-nitrate (28-0-0) at the recommended rate for corn following corn at each location and plots receiving the CoRoN® treatment received 11.2 kg ha^{-1} less than all other plots to adjust for the N contribution from the foliar-applied N (Table 2-2). Dates of all field activities are listed in Table 2-3.

Table 2-2. Starter fertilizer analysis and rate and sidedress fertilizer analysis and rate used at each of the locations to examine the response of corn to V5 and R1 applications of fungicides and foliar fertilizer (V5 only) in 2012 and 2013.

Location	Starter Fertilizer		Sidedress Fertilizer	
	Analysis	Rate (kg N ha^{-1})	Analysis	Rate† (kg N ha^{-1})
ACRE	19-17-0	24	28-0-0	252
DPAC	19-17-0	34	28-0-0	258
NEPAC	28-0-0	31	28-0-0	213
PPAC	19-17-0	26	28-0-0	246
SEPAC	22-11-0	45	28-0-0	190

† Sidedress fertilizer rate was 11.2 kg ha^{-1} less than the value shown in plots receiving CoRoN® to compensate for the N applied in this material.

Table 2-3. Dates of all field activities for the 2012 and 2013 growing season.

	Location							
	2012			2013				
	ACRE	PPAC	SEPAC	ACRE	DPAC	NEPAC	PPAC	SEPAC
Planting	19-Apr	10-May	26-Apr	20-May	14-May	14-May	7-May	16-May
Disease Rating (V5)	28-May	4-Jun	24-May	17-Jun	11-Jun	18-Jun	19-Jun	10-Jun
Crop Reflectance (V5)	29-May	4-Jun	24-May	17-Jun	11-Jun	18-Jun	19-Jun	10-Jun
Treatment Applications (V5)	30-May	5-Jun	25-May	18-Jun	12-Jun	19-Jun	20-Jun	11-Jun
Side-dressed	30-May	8-Jun	25-May	18-Jun	4-Jun	17-Jun	21-Jun	3-Jun
Canopy Temperature Readings (Post V5)	n/a†	n/a	n/a	26-Jun	25-Jun	27-Jun	1-Jul	24-Jun
Disease Rating (Post V5)	21-Jun	28-Jun	18-Jun	5-Jul	8-Jul	2-Jul	1-Jul	3-Jul
Crop Reflectance (Post V5)	21-Jun	28-Jun	18-Jun	n/a‡	8-Jul	9-Jul	1-Jul	3-Jul
Disease Rating (R1)	8-Jul	18-Jul	5-Jul	23-Jul	18-Jul	25-Jul	18-Jul	16-Jul
SPAD Readings (R1)	8-Jul	18-Jul	5-Jul	23-Jul	18-Jul	25-Jul	18-Jul	16-Jul
Treatment Applications (R1)	9-Jul	23-Jul	6-Jul	24-Jul	19-Jul	26-Jul	19-Jul	17-Jul
Disease Rating (R5)	1-Aug	14-Aug	6-Aug	26-Aug	19-Aug	2-Sep	9-Sep	30-Aug
SPAD Readings (R5)	1-Aug	14-Aug	6-Aug	26-Aug	19-Aug	2-Sep	9-Sep	30-Aug
Green Leaf Rating (R5)	1-Aug	14-Aug	6-Aug	26-Aug	19-Aug	2-Sep	9-Sep	30-Aug
Ear Sample Collection (R6)	19-Sep	10-Oct	13-Sep	8-Oct	21-Sep	27-Sep	4-Oct	20-Sep
Push Test (R6)	19-Sep	10-Oct	13-Sep	8-Oct	21-Sep	27-Sep	4-Oct	20-Sep
Harvested	1-Oct	12-Oct	19-Sep	24-Oct	5-Nov	21-Oct	14-Oct	4-Oct

† n/a = not available. Temperatures were not measured during the 2012 growing season.

‡ n/a = not available. Saturated soils and wind damage prior to tasseling prevented the collection of reflectance data.

2.2.1 Treatments

A randomized complete block design was used at all locations in 2012 and 2013 to assess the effects of 7 treatments. The number of replicates was 3 (DPAC, SEPAC), 4 (ACRE, NEPAC), and 5 (PPAC). Individual plots were 9.1 meters wide, consisting of 12 rows of corn spaced 76 cm apart. Plot length was 70 m (ACRE), 335 m (DPAC), 120 m (NEPAC), 140 m (PPAC), and 610 m (SEPAC).

Treatments of Headline® [pyraclostrobin: (carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester)] by BASF Corporation (Florham Park, NJ), Quadris® [azoxystrobin: (methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate)] by Syngenta Crop Protection, Inc. (Greensboro, NC), Stratego YLD® [(10.8% prothioconazole: (2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,3-triazole-3-thione)) and (32.3% trifloxystrobin: ((E,E)-alpha-(methoxyimino)-2-[[[[1-[3-(trifluroromethyl)phenyl] ethylidene]amino]oxy]methyl]-, methylester)] by Bayer CropScience LP (Research Triangle Park, NC), and CoRoN® [25% total N (18.8% urea N, 6.2% methylene diurea and methylene urea), 0.5% B] by Helena Chemical Company (Collierville, TN) were targeted to be applied when plants were at V5 and were actually applied between V5 and V7. Applications of Headline AMP® [(13.6% pyraclostrobin: (carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester)) and (5.1% metconazole: (5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol))] by BASF corporation (Research Triangle Park, NC) were targeted to be made at R1 and were actually applied between R1 and R2.

Treatment details are summarized in tables 2-4, 2-5, and 2-6. Treatments will be referred to by their abbreviation (Table 2-4) throughout the rest of the paper.

Table 2-4. Targeted growth stages, active ingredients, and rates at which foliar fungicides and a foliar fertilizer were applied at all locations during 2012 and 2013 growing seasons. Treatment abbreviations used throughout the rest of the paper are listed below.

Treatment	Growth Stage	Abbreviation	Rate	Active Ingredient
Control		Control	None	None
CoRoN	V5	V5-C	11.2 kg ha^{-1}	Nitrogen
			0.2 kg ha^{-1}	Boron
Headline	V5	V5-H	99 g a.i. ha^{-1}	Pyraclostrobin
Quadris	V5	V5-Q	96 g a.i. ha^{-1}	Azoxystrobin
Stratego YLD	V5	V5-S	15 g a.i. ha^{-1}	Prothioconazole
			45 g a.i. ha^{-1}	Trifloxystrobin
Headline AMP	R1	R1-H	95 g a.i. ha^{-1}	Pyraclostrobin
			36 g a.i. ha^{-1}	Metconazole
Headline + Headline AMP	V5 + R1	V5R1-H	†	

† Headline + Headline AMP treatment received the same rates of Headline and Headline AMP as listed above.

Table 2-5. Actual corn growth stages at the time of the specific field activities.

	Location							
	ACRE	PPAC	SEPAC	ACRE	DPAC	NEPAC	PPAC	SEPAC
	2012			2013				
Disease Rating (V5)	V6	V5	V5	V5	V5	V6	V7	V5
Crop Reflectance (V5)	V6	V5	V5	V5	V5	V6	V7	V5
Treatment Applications (V5)	V6	V5	V5	V5	V5	V6	V7	V5
Leaf Temperature (Post V5)	n/a†	n/a	n/a	V8	V10	V8	V10	V7
Disease Rating (Post V5)	V12	V11	V9	V10	V14	V10	V10	V10
Crop Reflectance (Post V5)	V12	V11	V9	n/a‡	V14	V14	V10	V10
Disease Rating (R1)	R1	R2	R1	R1	R1	R1	R1	R1
SPAD Readings (R1)	R1	R2	R1	R1	R1	R1	R1	R1
Treatment Applications (R1)	R1	R2	R1	R1	R1	R1	R1	R1
Disease Rating (R5)	R5	R5	R5	R5	R5	R5	R5	R5
SPAD Readings (R5)	R5	R5	R5	R5	R5	R5	R5	R5
Green Leaf Rating (R5)	R5	R5	R5	R5	R5	R5	R5	R5
Ear Sample Collection (R6)	R6	R6	R6	R6	R6	R6	R6	R6
Push Test (R6)	R6	R6	R6	R6	R6	R6	R6	R6

† n/a = not available. Temperatures were not measured during the 2012 growing season.

‡ n/a = not available. Saturated soils and wind damage prior to tasseling prevented the collection of reflectance data.

Table 2-6. Application pressure, nozzle size, nozzle spacing, carrier volume, and application equipment used to apply CoRoN and fungicide treatments to corn at V5 and R1.

Spray Timing		Location							
		2012			2013				
	Category	ACRE	PPAC	SEPAC	ACRE	DPAC	NEPAC	PPAC	SEPAC
V5	Pressure (kPa)	207	276	276	207	345	414	207	276
	Nozzle	8002	11008	11006	8002	8006	11006	11006	11006
	Nozzle spacing (cm)	51	38	38	51	51	38	38	38
	Carrier (L ha^{-1})	187	187	187	187	187	187	187	187
	Applicator	Tractor†	Miller	Apache	Tractor	Spra-Coupe	Spra-Coupe	Miller	Apache
R1	Pressure (kPa)	276	276	276	276	276	345	207	138
	Nozzle	11004	11008	11006	11004	11006	11005	11006	8006
	Nozzle spacing (cm)	38	38	38	38	38	38	38	38
	Carrier (L ha^{-1})	281	187	187	281	337	262	187	187
	Applicator	Hagie	Miller	Hagie	Hagie	Hagie	Hagie	Miller	Hagie

† A homemade tractor mounted spray tank was used to apply treatments.

‡ Miller® - Miller STN, St. Nazianz, WI; Apache® - Equipment Technologies, Mooresville, IN; Spra-Coupe® - AGCO Corporation, Duluth, GA; Hagie® - Hagie Manufacturing Company, Clarion, IA.

2.2.2 Disease Rating

Diseased leaf area was visually rated four times during plant development at each location during both growing seasons. Ratings were made on the same thirty consecutive plants in each plot throughout the growing season, which were chosen to be representative of all plants within the plot. Multiple sampling transects were used at PPAC (2), NEPAC (2), DPAC (3), and SEPAC (3) because of their greater plot lengths than ACRE (1). Walkways to enable plot access were tilled perpendicular to the rows approximately 4.5 meters from the plants that were rated for disease. Multiple individuals conducted disease ratings throughout the growing season, but each individual worked in separate transects to eliminate the effects of different disease raters on treatment comparisons within a replication. Samples were submitted to a diagnostic laboratory for confirmation of disease symptoms.

The first disease rating was targeted to take place at V5 prior to the application of the V5 treatments. Disease was rated as a percentage of leaf area affected on the two leaves above the first true leaf, and the average percentage between the two leaves was recorded (K.A. Wise, personal communication). Anthracnose leaf blight (*Colletotrichum graminicola* Ces.) was the only disease detected at this stage at all locations during both growing seasons.

Plants were rated for disease again at about V10, approximately three weeks after the V5 application. Since there was a lack of substantial disease development in the 2012 growing season, plants were rated for total percentage of leaf area damaged by both abiotic and biotic sources. All discoloration, including lesions, nutrient deficiencies, mechanical damage, etc. was recorded in 2012, regardless of its origin. The total number

of leaves with any type of discoloration on each plant was recorded as well (Wise, personal communication). Conditions were more conducive for disease development in 2013, so the ratings were based on the average percentage of diseased leaf area specifically caused by foliar fungal pathogens (Table 2-7).

Table 2-7 Diseases observed in 2013 at the V10 rating.

Location	Disease	Latin Name
ACRE	Anthracnose leaf blight (ALB)	*Colletotrichum graminicola*
DPAC	Gray leaf spot (GLS)	*Cercospora zeae-maydis*
NEPAC	Anthracnose leaf blight (ALB)	*Colletotrichum graminicola*
	Gray leaf spot (GLS)	*Cercospora zeae-maydis*
	Northern corn leaf blight (NCLB)	*Exserohilum turcicum*
PPAC	Anthracnose leaf blight (ALB)	*Colletotrichum graminicola*
SEPAC	Anthracnose leaf blight (ALB)	*Colletotrichum graminicola*

Disease ratings were also made at about R1 shortly before the R1 treatment applications. Disease was rated based on incidence (Munkvold, 1997). The earleaf and the three leaves below the earleaf were observed for the presence of disease. If one lesion was visible on any of the four leaves, a "yes" rating was given for that particular plant. From this, the percentage of the thirty plants that received a "yes" rating was calculated. In 2013 at the NEPAC location, the percentage of diseased earleaf area was recorded due to 100% incidence of disease on the earleaf across all plots.

The final disease rating was conducted on plants when corn had reached the R5 (kernel dent) stage of kernel development. Visual ratings for disease severity were recorded as the percentage of earleaf area affected by disease (Byamukama et al., 2013).

Gray leaf spot was the primary disease observed at all locations during 2012 and 2013, with the exception of NEPAC and DPAC in 2013, where NCLB was observed in addition to GLS.

2.2.3 Canopy Reflectance

Crop canopy reflectance data were collected along the entire plot length at all locations with two models of active optical reflectance sensors, two Crop Circle™ model ACS-470 (Holland Scientific, Inc., Lincoln, NE) sensors and two Greenseeker® model RT200 (NTech Industries, Inc., Ukiah, CA) sensors. In 2012, the Crop Circle sensor was equipped with blue (450 nm), red-edge (730 nm), and NIR (760 nm) filters. After receiving new filters in 2013, the blue filter was replaced with a green filter (550 nm) due to green being more sensitive to changes in pigment concentration (Hatfield et al., 2008). The Greenseeker sensor measured red (650 nm) and NIR (780 nm) canopy reflectance by default. Vegetative indices used for analysis are based on the following equations:

Chlorophyll Index (Ciganda et al., 2008):

$$CI = (NIR/PAR)-1$$

Normalized Difference Vegetative Index (Gitelson et al., 1996):

$$NDVI = (NIR-PAR)/(NIR+PAR)$$

The manufacturers recommended that the optical sensors be positioned between 76 and 90 cm (Crop Circle) and between 71 and 122 cm (Greenseeker) above the crop canopy. All four sensors were mounted on a hydraulically operated front boom of a modified high-clearance spray applicator (sensor buggy) and maintained at a constant height of 80 cm above the crop canopy. The Greenseeker was mounted directly in front

of the Crop Circle, and the pairs of sensors were centered above individual rows of corn spaced 76 cm apart. Optical reflectance data were collected at speeds ranging from 6.5 to 9.5 km hr^{-1}, and measurements were made on two of the center 6 rows of each 12-row plot.

Each pass across the field was geo-referenced with a Trimble® Ag132 GPS receiver that utilized an OmniSTAR® VBS DGPS service (OmniSTAR, Houston, TX). Individual wavelength data was recorded with a Geo Scout™ GLS-420 data logger (Holland Scientific, Inc., Lincoln, NE) for the Crop Circle and a Trimble TDS Nomad (Trimble Navigation Limited, Corvallis, OR) for the Greenseeker.

Crop reflectance was measured prior to V5 treatment applications to establish the variability in baseline reflectance among plots. Crop reflectance was collected again around V10 to quantify V5 treatment effects. Crop reflectance could not be measured after R1 applications due to insufficient clearance of the sensor buggy.

2.2.4 SPAD

Minolta SPAD™ 502 (Konica Minolta Optics, Inc., Tokyo, Japan) chlorophyll meters were used to quantify leaf greenness immediately before the R1 applications and again at R5 (kernel dent). Readings were taken on the same thirty consecutive plants that were rated for disease in each transect in each plot. Before use, each SPAD meter was calibrated with the factory-supplied calibration filters to ensure that each meter was reading within specifications. The same SPAD meter was used either in one transect (all replicates) or within an entire replicate to eliminate potential variability due to differences in SPAD meters. Each reading with the SPAD meter was taken midway between the leaf

sheath and leaf tip and midway between the midrib and edge of the leaf. Before the R1 treatments, readings were taken on the earleaf of each plant, and the average value from the thirty plants was recorded. At R5, readings were taken on the earleaf and the second leaf above the earleaf.

2.2.5 Green Leaf Index

A visual rating of leaf greenness was conducted on the same 30 plants at R5 in a similar fashion as that described by Binford and Blackmer (1993). Individual leaves below the earleaf (not including the earleaf) were given a rating of 0, 0.25, 0.5, 0.75, or 1 based on the relative proportion of green area still visible on the leaf (0 = no green area). The values from each of the remaining green leaves below the earleaf were summed to give an overall green leaf index for the plant.

2.2.6 Leaf Surface Temperature

ThermoWorks IR-Pro-USB (ThermoWorks, Lindon, Utah) infrared thermometers were used in 2013 to measure leaf surface temperature on the same thirty plants described previously. Leaf temperatures were targeted to take place within two weeks of the V5 treatment applications, which is within the average residual period of 21 days for foliar fungicides (Balba, 2007). Each temperature reading was taken midway between the leaf sheath and leaf tip of the most recently fully collared leaf. The thermometers were pointed in a 90-degree angle approximately 15 cm from the leaf surface. Data were collected early afternoon when the plants were dry and temperatures were warmer.

2.2.7 Weather Data

Weather data (daily maximum temperature, daily minimum temperature, and daily precipitation) were recorded by automated weather stations in close proximity to each field experiment and data were obtained from the Indiana State Climate Office (http://iclimate.org/index.asp). Relative humidity data were obtained from the National Oceanic and Atmospheric Administration (www7.ncdc.noaa.gov/IPS) from weather stations located in Indianapolis, South Bend, and Fort Wayne, IN.

2.2.8 Push Test

A push test similar to that described by Malvick and Nicolai (2005) was conducted in each plot at R6 (physiological maturity) on the same thirty plants that previous ratings were taken, to determine whether treatments had any effect on stalk strength. Plants were pushed approximately 30 cm horizontally at ear height. If stalks broke or remained bent over, they received a "yes" rating for stalk lodging. The number of plants receiving a "yes" rating out of 30 was and converted to a percentage for the sample.

2.2.9 Yield Components

Ears were collected for yield component analysis from each location after physiological maturity (R6) but prior to harvest. Eighteen ears were collected in evenly spaced intervals across the entire plot length at ACRE, DPAC, NEPAC, and PPAC. Due to variability in drought severity across plots at SEPAC, 15-ear samples were collected at each of the three transects where disease ratings took place across plots. The 15-ear

samples were collected from every other plant starting at the flag marking plants for disease rating.

Rows of kernels around the ear and kernels row^{-1} were counted to determine the total number of kernels ear^{-1}. Due to drought conditions during the 2012 growing season, many of the ears collected were poorly pollinated as a whole or had missing rows ["zipper" ears; as described by Nielsen, (2011)]. Each individual kernel was counted and recorded for the poorly pollinated ears, so rows ear^{-1} and kernels row^{-1} were not counted. Only the rows of kernels and kernels row^{-1} that were present on each ear was recorded.

Ears were then shelled, the grain thoroughly mixed by hand, and approximately 1500 kernels were taken out of the sample with a measuring cup. Kernels were then placed on a sieve with 0.635 cm holes to remove cracked kernels, excessively small kernels, and chaff. Two 500 kernel samples were counted with a Key-Mat® Model 946 seed counter (Key-Mat Equipment Co., Inc, Batavia, IL) and placed in a forced air oven at 60˚C until dried to a constant weight. Samples were then weighed on an analytical scale to determine thousand-kernel dry weight (TKW).

The middle 6 rows of each 12 row plot were mechanically harvested with 6-row combines equipped with calibrated Ag Leader® yield monitors (PF3000, PFAdvantage, or Integra models depending on location) to estimate grain yield and harvest moisture.

2.2.10 Spatial Data Processing

Spatial data from active optical reflectance sensors and yield monitors were imported into ArcGIS® 10.1 (ESRI, Redlands, CA) and intersected with plot layers. Individual data points within approximately 15 m of plot ends were removed to ensure

sensors were centered over the rows after making turns and that grain flow had reached a constant flow rate in the combine. Unusually high or low anomalous values were typically removed from the dataset. These extremes were commonly removed by selecting problem areas in the field or by selecting values above or below two standard deviations from the mean.

2.2.11 Grain Nutrient Analysis

Approximately 500 mL of grain were ground to a fine powder using a food processor and 100 mL samples were sent to A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN) for nutrient analysis. Samples were analyzed for nitrogen (N), sulfur (S), phosphorus (P), potassium (K), magnesium (Mg), boron (B), zinc (Zn), manganese (Mn), and iron (Fe). The Dumas Method (AOAC, 1995) was used for determining total N. This process involves subsequent combustions at 850-900˚C to free N before being measured. Concentrations of all other minerals were determined by the Inductively Coupled Plasma Spectroscopic Method (AOAC, 1995).

2.2.12 Statistical Analysis

All visual assessments of disease incidence, disease severity, and stalk rot ratings were arcsine transformed prior to analysis (Sokal and Rohlf, 1981). Analysis of variance was performed on all data for this randomized complete block experiment using PROC GLM of SAS version 9.3 (SAS Inst., Cary, NC). *A priori* single-degree-of-freedom contrasts were used to compare different timings of fungicide applications to the control and to compare treatments of specific interest. Mean separation tests were performed

using Fisher's Protected Least Significant Difference ($P \leq 0.05$). Site-years were not combined because most variables failed to meet the homogeneity of variance requirement when subjected to Bartlett's test of homogeneity ($P > 0.01$). Where possible (F-prime non-significant at $P > 0.01$), we combined years within a location. Years were considered to be a random effect. The year by treatment interaction was not significant ($P > 0.25$) for the majority of the parameters, so we pooled the year by treatment interaction with experimental error in all cases. Mean separation tests and contrasts were performed on transformed units, but results are presented in backtransformed units. The coefficient of variation and least significant difference value were not presented for transformed units. A combined year analysis was not considered for the V10 disease assessments (number of leaves and disease severity) due to a difference in rating procedure between 2012 and 2013. Where years were not combined, results are presented within location and year (e.g., ACRE '12 and ACRE '13 for variable grain yield).

2.3 Results and Discussion

2.3.1 Weather Conditions

In 2012, soil and weather conditions (Fig. 2-1) were favorable for planting ACRE and SEPAC around the middle of April. Rainfall in April delayed planting until early May at PPAC (Fig. 2-1). Very few precipitation events occurred after planting at all three locations (Fig. 2-1), and the lack of precipitation continued throughout the majority of the growing season resulting in limited soil water availability for adequate crop growth and development. By the first week of July, cumulative precipitation was approximately 200 mm lower than the 30-year average (1981-2010) cumulative precipitation (Fig. 2-1). The lack of precipitation likely contributed to the poor pollination and kernel abortion at SEPAC and ACRE. Small, but more frequent, precipitation events at PPAC (Fig. 2-1) likely resulted in less stress during pollination and grain fill compared to the other two locations. Average daily temperatures tended to be much higher than the 30-year average daily temperatures for the majority of the growing season at all three locations (Fig. 2-1). Conditions were not favorable for foliar disease development during the 2012 growing season because of the above average temperatures, below average precipitation, and below average relative humidity (Figs. 2-1 and 2-2). Many plant pathogens require high humidity (Bergstrom and Nicholson, 1999; Levy and Pataky, 1992; Ward et al., 1999).

In contrast, multiple early-season precipitation events occurred in 2013, which delayed planting until the middle of May at all five locations (Figs. 2-3 and 2-4). Overall, once the crops were planted, the 2013 growing season proved to be much closer to the 30-year average than 2012. Cumulative precipitation was very similar to the 30-year average (1981-2010) cumulative precipitation in central Indiana at ACRE and DPAC

(Figs. 2-3 and 2-4). Cumulative precipitation was above the 30-year average in the northern part of the state at NEPAC and PPAC (Figs. 2-3 and 2-4). Very few precipitation events occurred after the first week in July at SEPAC (Fig. 2-4), resulting in drought stress conditions during the grain filling period. The average daily temperature was similar to the 30-year average daily temperature at all locations (Figs. 2-3 and 2-4). Conditions were favorable for foliar disease development in June when temperatures were near average and relative humidity was higher than average (Figs. 2-2, 2-3, and 2-4). However, a sudden drop in temperatures around the beginning of July and again at the beginning of August likely slowed disease progression.

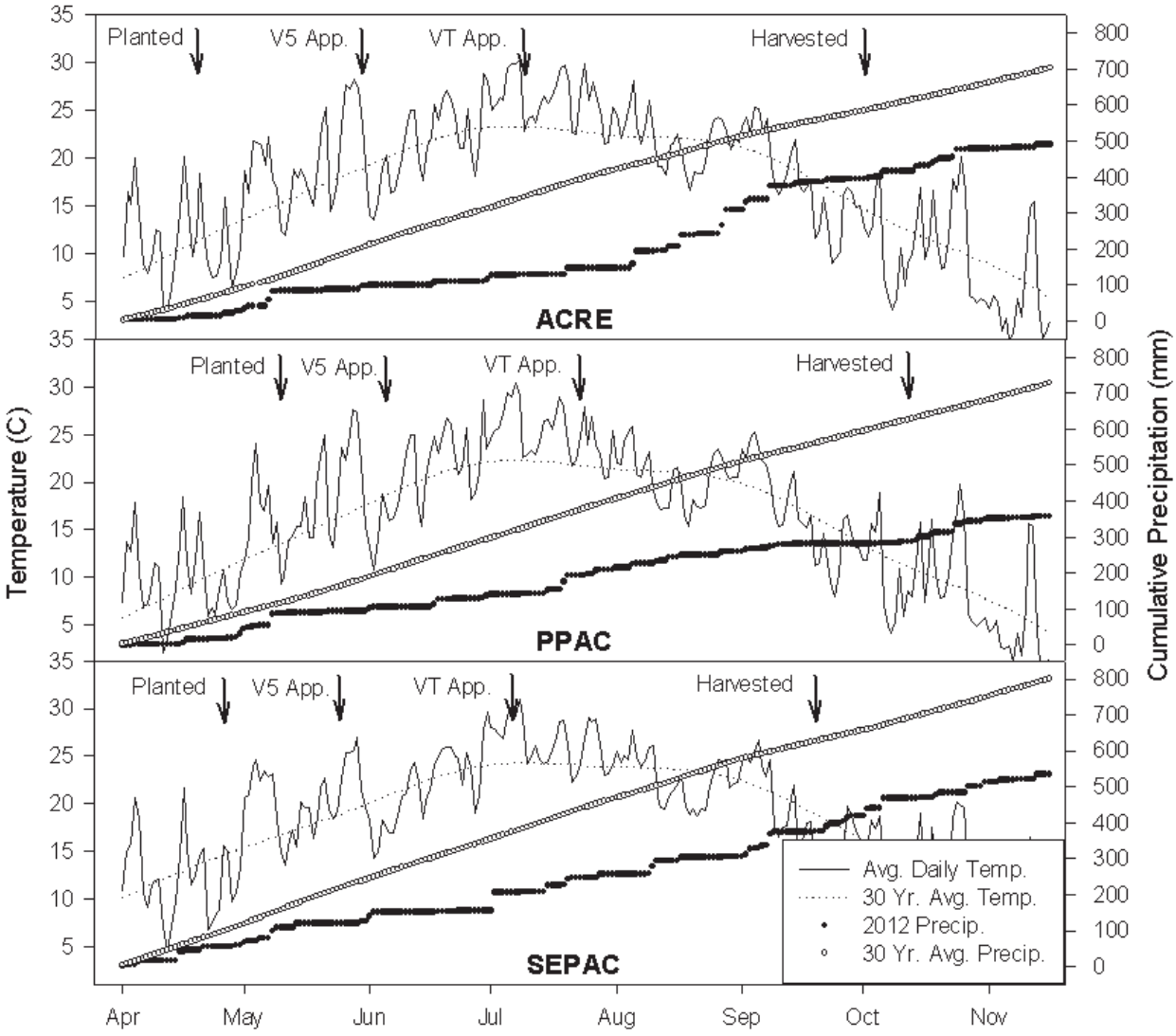

Figure 2-1. Average daily temperature and cumulative precipitation for the 2012 and the 30-year average (1981-2010) growing season at ACRE, PPAC, and SEPAC. Data obtained from the Indiana State Climate Office (http://iclimate.org/index.asp).

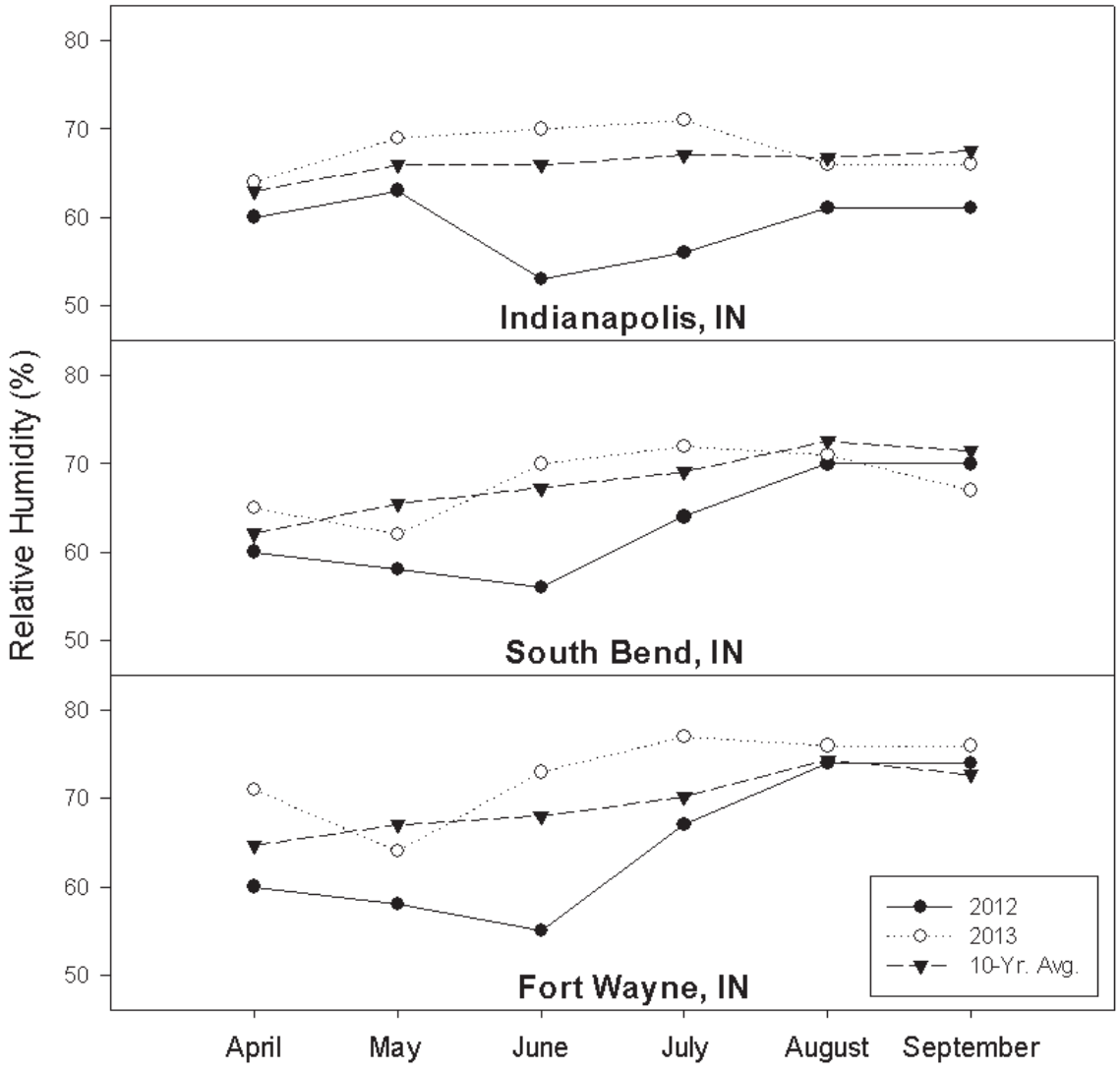

Figure 2-2. Monthly relative humidity from selected weather stations across Indiana for 2012, 2013, and the 10-year average (2004-2013) growing season. Data obtained from the National Oceanic and Atmospheric Administration (www7.ncdc.noaa.gov/IPS).

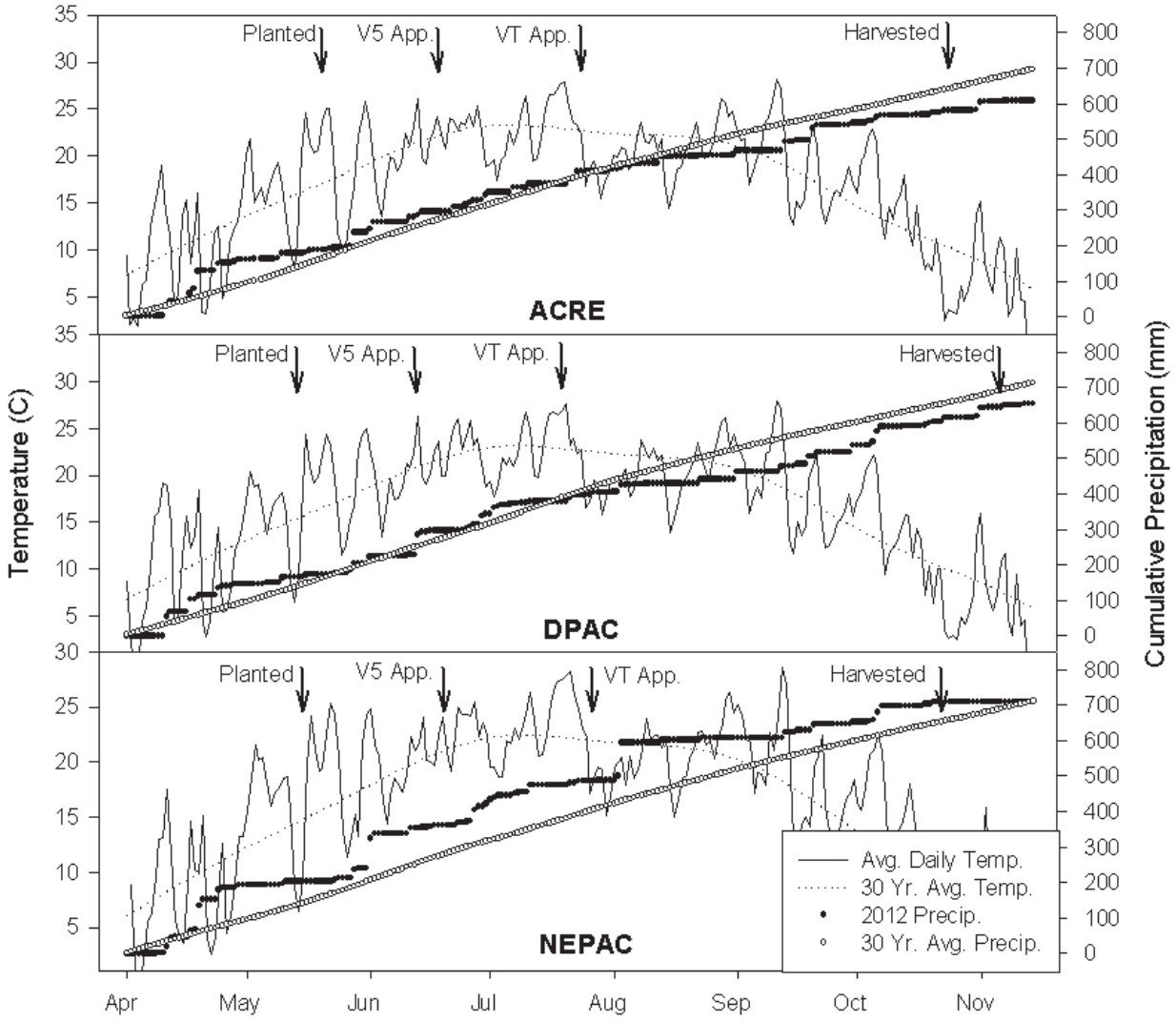

Figure 2-3. Average daily temperature and cumulative precipitation for the 2013 and the 30-year average (1981-2010) growing season at ACRE, DPAC, and NEPAC. Data obtained from the Indiana State Climate Office (http://iclimate.org/index.asp).

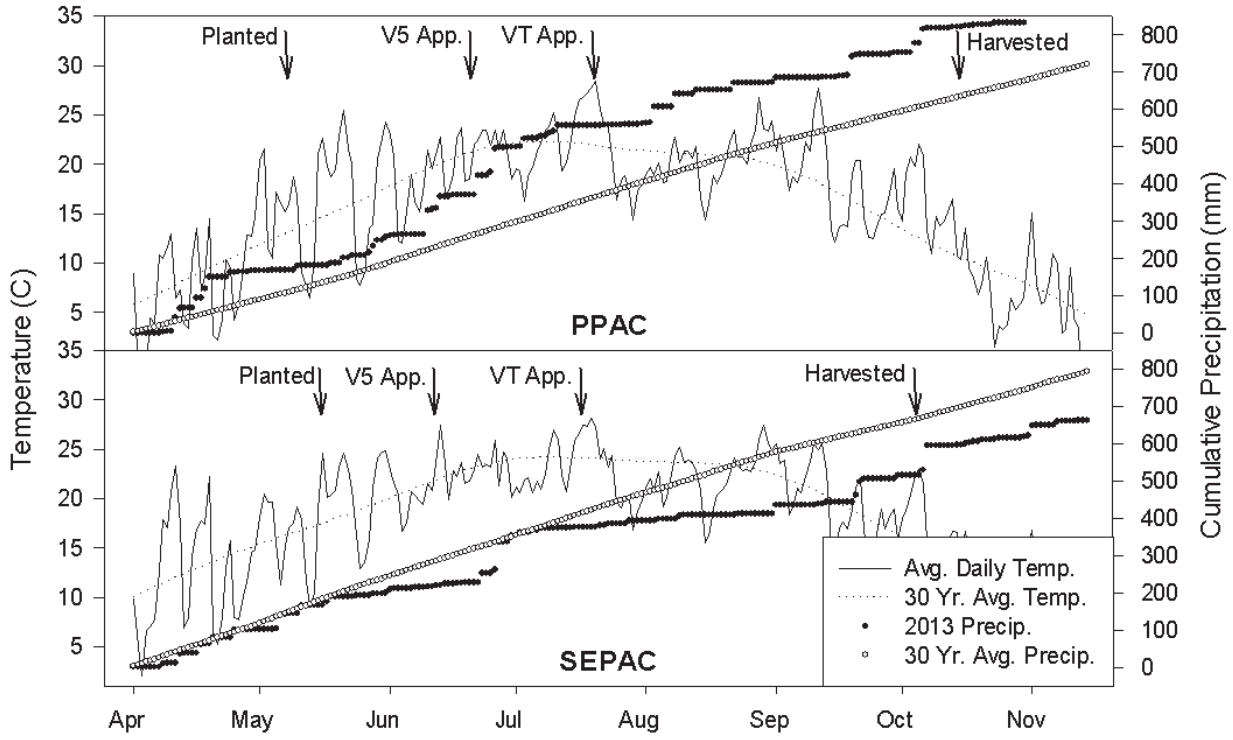

Figure 2-4. Average daily temperature and cumulative precipitation for the 2013 and the 30-year average (1981-2010) growing season at PPAC and SEPAC. Data obtained from the Indiana State Climate Office (http://iclimate.org/index.asp).

2.3.2 Pre-Treatment Disease Ratings and Canopy Reflectance

No differences in ALB severity were observed at V5 prior to foliar treatment applications at any of the locations except PPAC (2-year mean; Table 2-8). However, differences in ALB severity between the control and R1-H plots at PPAC were unlikely to have a differential effect on plant growth and development (0.09% for the control vs. 0.02% for the R1-H treatment) and were not evident in the subsequent disease rating at V10 (Table 2-11). No differences in crop canopy reflectance were observed at any of the locations prior to the V5 treatment applications (Table 2-9). This suggests that up to the

time of V5 treatments, there were no apparent underlying differences among the plots for the incidence and severity of foliar leaf diseases or for canopy biomass and health (canopy reflectance).

Table 2-8. Percentage of leaf area affected by anthracnose leaf blight (*Colletotrichum graminicola*) at V5 prior to application of foliar treatments. Future treatments were to be applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were to be applied at R1 as Headline AMP (R1-H). One treatment was to receive Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year					
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12	SEPAC '13
	Anthracnose leaf blight, % Severity					
Control	0.08	0.02	0.7	0.09 a†	0.01	0.02
V5-C	0.13	0.02	0.8	0.07 a	0.04	0.02
V5-H	0.12	0.01	0.7	0.06 a	0.01	0.01
V5-Q	0.09	0.02	0.8	0.07 a	0.01	0.01
V5-S	0.08	0.02	0.7	0.10 a	0.01	0.02
V5R1-H	0.08	0.01	0.7	0.05 ab	0.01	0.03
R1-H	0.12	0.02	0.8	0.02 b	0.01	0.02
Grand Mean	0.10	0.02	0.7		0.01	0.02
Level of Significance	0.3	0.1	0.4	**0.02**	0.9	0.5

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-9. Effect of fungicides and a foliar fertilizer applied at V5 on crop canopy reflectance measured by Crop Circle [PAR wavelengths of 450 (2012), 550 (2013), and 730 nm and NIR wavelength of 760 nm] and Greenseeker (PAR wavelength of 650 nm and NIR wavelength of 780 nm) active optical reflectance sensors averaged across two growing seasons. No treatment effects were observed ($P \leq 0.05$), so the grand mean of all reflectance data, both pre- and post- treatment application from each location is presented. Treatments were applied as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S).

	Location									
	ACRE		DPAC†		NEPAC†		PPAC		SEPAC	
	Pre-Treatment (V5)									
Vegetative Index‡	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
CI450†	3.04	5	n/a§	n/a	n/a	n/a	1.86	7	1.98	2
CI550†	1.54	5	1.19	4	1.54	4	2.63	3	1.25	5
CI730	0.54	7	0.40	6	0.57	6	0.71	3	0.34	6
CI650	1.57	10	0.85	9	1.59	6	3.02	6	0.83	8
NDVI450†	0.59	2	n/a	n/a	n/a	n/a	0.48	4	0.50	1
NDVI550†	0.43	3	0.37	3	0.43	2	0.57	1	0.38	3
NDVI730	0.21	6	0.16	5	0.22	4	0.25	2	0.15	4
NDVI650	0.43	6	0.29	6	0.43	3	0.51	2	0.29	5
	Post-Treatment (V10)									
Vegetative Index	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
CI450†	9.31	7	n/a	n/a	n/a	n/a	15.34	9	11.02	11
CI550†	n/a	n/a	4.64	3	4.91	5	4.35	7	4.73	1
CI730	1.08	4	1.88	2	1.92	5	1.58	4	1.45	2
CI650	5.31	6	12.90	6	11.40	4	10.31	6	7.64	7
NDVI450†	0.80	1	n/a	n/a	n/a	n/a	0.86	1	0.81	1
NDVI550†	n/a	n/a	0.70	1	0.71	2	0.68	2	0.70	<1
NDVI730	0.35	2	0.48	1	0.49	2	0.44	2	0.41	1
NDVI650	0.71	2	0.87	1	0.85	1	0.83	1	0.77	2

† Represents data from one growing season only.

‡ Vegetative indices are calculated as: NDVI = (NIR-PAR)/(NIR+PAR) and CI = (NIR/PAR) – 1.
For example: CI450 = (760/450)-1.

§ n/a = not available. Data is missing as a result of changing wavelength filters between years and the inability to take post-treatment reflectance data at ACRE in 2013.

¶ See Table 2-4 for active ingredients and application rates of treatments.

2.3.3 Leaf Canopy Temperature

Minimal research has been reported on the effects of foliar fungicides on crop canopy temperature. Some studies have indicated that strobilurin fungicides cause a decrease in stomatal conductance leading to a decrease in transpiration rates (Grossman et al., 1988; Nason et al., 2007). Thus, one could speculate that canopy temperatures may increase slightly after the application of strobilurin fungicides, but neither study included canopy temperature measurements. In wheat (*Triticum aestivum* L.) with no disease present, canopy temperature increased one day after application of pyraclostrobin in a controlled environment, but ultimately decreased relative to the non-treated control a few days later (Inagaki et al., 2009). Pyraclostrobin depressed root water uptake, resulting in slowed soil drying which may have caused the differences in leaf temperature (Inagaki et al., 2009). However, no differences in temperature were detected in a similar field experiment (Inagaki et al., 2009).

In this study, none of the V5 foliar treatments affected leaf canopy temperature as measured approximately 1.5 to 2 weeks after the V5 applications (Table 2-10), which is within the average residual period of strobilurin fungicides, approximately 21 days (Balba, 2007). Canopy temperatures varied among locations from 15.5 to 25.2˚C (Table 2-10) and ambient air temperatures varied among locations from 25.6 to 33.9˚C (data not shown). On average, plant canopy temperatures ranged from 3.8 to 16.2˚C lower than ambient air temperatures, averaging 9.4˚C lower than ambient air temperatures (data not shown). The absence of treatment effects on plant canopy temperature are in agreement with the field experiment conducted by Inagaki et al. (2009).

Table 2-10. Effect of foliar fungicides and a foliar fertilizer applied at V5 on canopy temperature in the 2013 growing season. Temperatures were taken on the most recently collared leaf with an infrared thermometer approximately 1.5 to 2 weeks after treatments were applied. Treatments were applied as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments not yet applied include Headline AMP at R1 (R1-H) and one treatment receiving both Headline at V5 and Headline AMP at R1 (V5R1-H).

	Location				
Treatment	ACRE	DPAC	NEPAC	PPAC	SEPAC
	---------- Canopy Temperature, ºC ----------				
Control	21.4†	26.4	23.4	15.9	21.4
V5-C	21.1	25.1	22.7	14.9	20.8
V5-H	22.0	25.9	22.5	15.9	21.2
V5-Q	22.5	26.0	23.0	14.8	20.8
V5-S	22.0	25.3	22.1	15.4	20.0
V5R1-H	22.0	24.1	21.4	15.7	21.2
R1-H	21.7	23.4	22.6	15.7	21.5
Grand Mean	21.8	25.2	22.5	15.5	21.0
Level of Significance	0.9	0.4	0.8	0.8	0.4
LSD (0.05)	ns	ns	ns	ns	ns
C.V.	4	4	4	4	2
Contrasts‡	---------- Level of Significance ----------				
Control vs. V5	0.4	0.6	0.4	0.5	0.2
Control vs. V5-C	0.8	0.4	0.6	0.3	0.4

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S.

§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.4 Post-V5 Application Disease Rating and Canopy Reflectance

Disease severity was too low to rate specific diseases in 2012, so foliar ratings for discoloration of any type were made that year. All discoloration, including lesions, nutrient deficiencies, mechanical damage, etc. was recorded, regardless of its origin. The V5 fungicide treatments had no effect on the percentage of such discolored leaf area in

2012 (Control vs. V5 contrasts; Table 2-11). In 2013, foliar ratings only included discoloration resulting from foliar fungal diseases. Collectively, the V5 fungicide treatments reduced disease severity in 2013 at DPAC and NEPAC (Control vs. V5 contrasts; Table 2-11). At DPAC, the V5-H and V5-S treatments were more effective at reducing disease severity than the V5-Q treatment (Table 2-11). No effects of V5-applied foliar fungicides were observed at the other locations; however, disease pressure was also very low when these ratings were made (approximately V10). Treatment "effects" at ACRE in 2012 and at PPAC in 2013 were relative to treatments that had not yet been applied and so represent non-treatment spatial variability among plots.

The total number of discolored leaves at V10 in 2012 was not affected by any treatments (Table 2-12). In 2013, V5 fungicide treatments reduced disease development in the plant canopy at 3 of the 5 locations (Table 2-12). The average number of diseased leaves for V5 fungicide treatments averaged over V5-H, V5-Q, and V5-S compared to the control was 0.6 vs. 2.8, 1.7 vs. 2.1, and 0.06 vs. 0.12 for DPAC, NEPAC, and SEPAC, respectively. Fewer diseased leaves were evident in the V5-H and V5-S treatments than the V5-Q treatment at DPAC (Table 2-12). No treatment effects were observed at the other locations.

No differences in crop canopy reflectance were observed at V10 in response to V5 foliar treatments at any of the locations (Table 2-9). Multiple studies have found strong correlations between canopy reflectance and LAI, biomass, and chlorophyll status (Filella and Penuelas, 1994; Gitelson et al., 2003; Houborg et al., 2009; Schlemmer et al., 2013; Solari et al., 2008). The crop canopy reflectance results of this study therefore suggest that fungicides applied at V5 did not affect LAI, biomass, or chlorophyll status.

The absence of any treatment effects on canopy reflectance at V10 also suggest that the small treatment effects on disease severity detected at V10 in 2013 (DPAC and NEPAC) had no effect on canopy reflectance.

Table 2-11. Effect of foliar fungicides and a foliar fertilizer applied at V5 on the percentage of leaf area discolored by any cause (2012) or damaged by foliar fungal diseases (2013) at about V10. Disease severity was too low in 2012 for specific disease ratings. Treatments were applied as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments not yet applied include Headline AMP at R1 (R1-H) and one treatment receiving both Headline at V5 and Headline AMP at R1 (V5R1-H).

	Location and Year							
Treatment	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12	PPAC '13	SEPAC '12	SEPAC '13
	% Severity at V10							
Control	0.17 abc†	0.03	0.54 a	0.84 ab	0.18	0.62 ab	2.7	0.04
V5-C	0.17 abc	0.02	0.35 b	0.82 ab	0.21	0.66 ab	1.6	0.04
V5-H	0.12 bc	0.01	0.09 c	0.51 c	0.22	0.58 ab	2.3	0.02
V5-Q	0.31 a	0.01	0.35 b	0.72 abc	0.14	0.68 a	1.5	0.02
V5-S	0.25 ab	0.02	0.08 c	0.55 bc	0.26	0.54 bc	1.6	0.03
V5R1-H	0.34 a	0.01	0.07 c	0.47 c	0.21	0.43 c	1.9	0.03
R1-H	0.07 c	0.01	0.42 ab	0.87 a	0.15	0.54 abc	1.6	0.06
Grand Mean		0.02			0.20		1.9	0.03
Level of Significance	**0.04**	0.3	**≤0.0001**	**0.03**	0.2	**0.02**	0.5	0.07
Contrasts‡	Level of Significance							
Control vs. V5	0.5	0.2	**≤0.0001**	**0.05**	0.5	0.7	0.1	0.1
Control vs. V5-C	1.0	0.4	**0.02**	0.9	0.5	0.6	0.1	0.8

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-12. Effect of foliar fungicides and a foliar fertilizer applied at V5 on the number of leaves discolored by any cause (2012) or by foliar fungal diseases (2013) at about V10. Disease severity was too low in 2012 for specific disease ratings. Treatments were applied as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments not yet applied include Headline AMP at R1 (R1-H) and one treatment receiving both Headline at V5 and Headline AMP at R1 (V5R1-H).

	Location and Year							
Treatment	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12	PPAC '13	SEPAC '12	SEPAC '13
	# of Leaves Affected at V10							
Control	0.3	0.06	2.8 a†	2.1	0.2	2.1	3.0	0.12 a
V5-C	0.3	0.04	1.4 c	1.8	0.3	2.0	2.9	0.11 ab
V5-H	0.2	0.04	0.2 d	1.6	0.3	2.1	2.5	0.06 cb
V5-Q	0.5	0.04	1.4 c	1.7	0.2	2.1	2.8	0.06 cb
V5-S	0.6	0.06	0.2 d	1.7	0.3	1.9	2.8	0.06 cb
V5R1-H	0.5	0.02	0.3 d	1.6	0.3	1.7	2.6	0.05 c
R1-H	0.2	0.04	2.0 b	2.1	0.2	2.0	3.1	0.13 a
Grand Mean	0.4	0.04		1.8	0.2	2.0	2.8	
Level of Significance	0.3	0.2	**≤0.0001**	0.06	0.2	0.1	0.1	**0.02**
LSD (0.05)	ns	ns	0.6	ns	ns	ns	ns	0.05
C.V.	77	55	28	16	45	12	7	34
Contrasts‡	Level of Significance							
Control vs. V5	0.4	0.4	**≤0.0001**	**0.02**	0.3	0.8	0.1	**0.01**
Control vs. V5-C	0.9	0.2	**0.0002**	0.8	0.3	0.6	0.6	0.6

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S.

§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.5 Disease Rating and Chlorophyll Meter Readings at R1

Disease incidence was the same at R1 without variance for a number of the treatments at ACRE and DPAC, thus statistical analyses were not performed and only mean disease incidence is presented (Table 2-13). Disease severity was recorded and analyzed at NEPAC only on the earleaf instead of the normal disease incidence rating (earleaf and three leaves below) because of the higher disease severity and 100% incidence of disease at this location. Although incidence of disease was near 100% at DPAC and ACRE in 2013 (Table 2-13), disease severity was not assigned on the earleaf because disease severity was extremely low on the earleaf at R1. The primary disease noted at this stage of development was GLS in 2012 and 2013. Northern corn leaf blight was observed in addition to GLS at NEPAC in 2013.

Fungicide treatments applied at V5 did not reduce disease severity on the earleaf at NEPAC at R1 (Control vs. V5 contrast; Table 2-13). At PPAC in 2013, disease incidence was lower for plots receiving the targeted V5 application of foliar fungicides (mean of 13%) than the control (98%; Control vs. V5 contrast; Table 2-13). The fungicides used in this study have a residual period of approximately 21 days (Balba, 2007). Based on an estimated residual period of 21 days, plants were unprotected by fungicides for approximately 15 days at NEPAC and 7 days at PPAC prior to R1 disease ratings. Since relative humidity was high at NEPAC and PPAC, the extended period of time without fungicidal activity at NEPAC as compared to PPAC before the subsequent R1 rating likely contributed to the lack of noticeable disease control at NEPAC compared to PPAC.

Previous studies have documented delayed senescence after fungicide applications (Diaz-Espejo et al, 2012; Gooding et al., 2000; Ruske et al., 2003; Pepler et al, 2005). The duration of green leaf life was extended after the application of strobilurin fungicides on grapevines, but the actual chlorophyll status was not increased compared to the untreated control as estimated by a SPAD chlorophyll meter (Diaz-Espejo et al., 2012).

In this study, leaf "greenness" at R1 and R5 were estimated using SPAD meters. The number of green leaves below the earleaf at R5 was also documented. No differences were observed for earleaf SPAD values at R1 between treatments at any of the locations (Table 2-14) and thus suggest that the foliar V5 treatments had no effect on leaf chlorophyll status. Chlorophyll meter values at R1 ranged from 51.7 to 57.3 (SPAD values) across locations (Table 2-14). Leaf N content and chlorophyll meter values have been shown to be highly correlated (Chapman and Barreto, 1997; Schepers et al., 1992) and yet this data suggests that neither the foliar fungicides nor the foliar fertilizer applied at V5 had any effect on earleaf N concentration.

Table 2-13. Effect of foliar fungicides and a foliar fertilizer applied at V5 on the percentage of earleaf area damaged by foliar fungal diseases at NEPAC and the incidence of disease at all other locations at R1. Treatments were applied as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments not yet applied include Headline AMP at R1 (R1-H) and one treatment receiving both Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year						
Treatment	ACRE† '12	ACRE† '13	DPAC† '13	NEPAC '13	PPAC '12	PPAC '13	SEPAC '12 & '13
	Incidence			% Severity	Incidence		
Control	0.2	100	100	1.3 b‡	2.8	98 ab	2.7
V5-C	0.2	100	99	1.1 b	4.9	91 b	0.9
V5-H	0.2	100	100	1.8 a	4.2	11 c	1.2
V5-Q	0.2	100	100	1.2 b	4.1	19 c	2.2
V5-S	0.0	97	100	1.4 ab	3.4	11 c	1.1
V5R1-H	0.0	89	100	1.9 a	4.8	8 c	0.9
R1-H	0.2	100	99	1.4 ab	3.4	100 a	1.2
Grand Mean	0.2	98	100		3.9		1.4
Level of Significance	n/a	n/a	n/a	**0.02**	0.9	**≤0.0001**	0.5
Contrasts§	Level of Significance						
Control vs. V5	n/a	n/a	n/a	0.2	0.4	**≤0.0001**	0.2
Control vs. V5-C	n/a	n/a	n/a	0.5	0.2	0.1	0.09

† Statistical analysis was not performed (n/a = not available) on incidence ratings at ACRE or DPAC because the majority of treatments had no variance in the data.

‡ Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

§ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S.

¶ See Table 2-4 for active ingredients and application rates of treatments

Table 2-14. Effect of foliar fungicides and a foliar fertilizer applied at V5 on SPAD chlorophyll meter values at R1. Treatments were applied as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments not yet applied include Headline AMP at R1 (R1-H) and one treatment receiving both Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year				
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	SPAD Units				
Control	51.8†	55.0	56.2	53.7	55.3
V5-C	52.7	55.5	55.2	54.2	55.2
V5-H	51.9	55.1	55.8	54.2	55.7
V5-Q	52.8	55.6	56.1	54.5	54.9
V5-S	51.7	55.2	56.4	54.0	54.6
V5R1-H	50.8	55.7	56.9	54.2	55.2
R1-H	51.7	56.4	57.3	54.4	55.1
Grand Mean	51.9	55.5	56.3	54.2	55.1
Level of Significance	0.2	0.3	0.09	0.7	0.8
LSD (0.05)	ns	ns	ns	ns	ns
C.V.	3	1	2	2	2
Contrasts‡	Level of Significance				
Control vs. V5	0.6	0.6	0.9	0.2	0.7
Control vs. V5-C	0.3	0.4	0.2	0.3	0.9

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).
‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S.
§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.6 Disease Rating, Chlorophyll Meter Readings, and Green Leaf Rating at R5

Disease severity on the earleaf at R5 was low (< 1%) at all the trials except for NEPAC (> 5%) (Table 2-15). Conventional tillage at ACRE, DPAC, and PPAC likely reduced the levels of primary inoculum at these locations and contributed to the low disease severity observed throughout the growing season. The tillage practice was no-till at both the NEPAC and SEPAC locations, which likely contributed to a greater amount of primary inoculum at the soil surface as compared to the locations with conventional

tillage. A hybrid rated as highly resistant to foliar fungal diseases was used at SEPAC and this location received very little rainfall during both 2012 and 2013, which likely contributed to the low disease severity (< 1%) throughout the growing season. However, at NEPAC, a hybrid rated as moderately resistant to foliar fungal diseases was used, and this location received average rainfall in 2013, which likely contributed to the higher disease severity (> 5%) than the other locations.

On average, applications of foliar fungicides near V5 resulted in lower earleaf disease severity at R5 (kernel dent stage) than the control at PPAC (2-year mean; Control vs. V5 contrast; Table 2-15). Environmental conditions were likely not as conducive for foliar disease development after the targeted V5 fungicide application residual period at PPAC, which was likely the reason disease severity was lower at R5. Treatment "effects" at SEPAC in 2013 for the V5 fungicide applications likely represent spatial variability among plots since no V5 fungicide treatment effects were observed at R1 (Control vs. V5 contrast; Tables 2-13 and 2-15).

Foliar fungicide treatments applied at R1 reduced earleaf disease severity compared to the control at all locations except for SEPAC in 2012 (Control vs. R1 contrast; Table 2-15). Mean disease severity was reduced from 0.8 to 0.3%, 0.9 to 0.6%, 7.5 to 6.2%, 1.0 to 0.6%, and 0.25 to 0.09% in response to foliar fungicides applied at R1 compared to the control at ACRE, DPAC, NEPAC, PPAC, and at SEPAC in 2013, respectively (Control vs. R1 contrast; Table 2-15). The R1 fungicide application timing was more effective at reducing disease severity than the V5 fungicide application timing at all locations (V5 vs. R1 contrast; Table 2-15). The V5R1-H treatment had lower levels of disease severity than the V5-H treatment at all locations, but there was no difference in

levels of disease severity between the R1-H and V5R1-H treatments (Table 2-15). This, again, indicates that the R1 fungicide application timing was more effective at reducing disease severity than the V5 timing, and it also indicates that two foliar fungicide applications at different times throughout the growing season did not reduce disease more than one fungicide application at R1.

No consistent differences for SPAD values were observed at R5 on the earleaf or the second leaf above the earleaf (Table 2-16). Differences observed on the earleaf were not always observed on the second leaf above the earleaf or vice versa (Table 2-16). SPAD values ranged from 43.2 to 53.2 and 45.3 to 54.3 across locations on the earleaf and the second leaf above the earleaf, respectively (Table 2-16). The R1 fungicide applications resulted in lower SPAD values compared to the control in both measured leaves at SEPAC (2-year mean), but higher SPAD values were observed in the second leaf above the earleaf at NEPAC (2013) [Control vs. R1 contrasts; Table 2-16]. As mentioned earlier, SPAD values were not increased compared to the untreated control on grapevines even though leaf senescence was delayed (Diaz-Espejo et al., 2012). Similarly, consistent increases in SPAD values in response to foliar fungicides were not detected. This suggests that fungicides did not have any effect on leaf tissue chlorophyll status or N status. The timing of the R5 measurements may have been too early to quantify differences in the rate of canopy senescence.

Fungicides applied at V5 increased the number of green leaves below the earleaf determined at R5 only at PPAC (2-year mean; Control vs. V5 contrast; Table 2-17). The number of green leaves present below the earleaf at R5 in response to R1 fungicide applications was on average 0.4 leaves greater than the control at DPAC (2013) and at

PPAC (2-year mean) [Control vs. R1 contrast; Table 2-17]. A previous study on corn also indicated that pyraclostrobin delayed senescence of the upper plant canopy even under low (≤ 5%) disease severity on the earleaf at R4 in some cases (Byamukama et al., 2013). The increase in green leaf area duration in low disease pressure situations in response to fungicides may be explained by a reduction in ethylene production under stressful conditions (Grossmann and Retzlaff, 1997) and fewer defense reactions induced by the plant (Bertelson et al., 2001).

Table 2-15. Effect of foliar fungicides and a foliar fertilizer on the percentage of earleaf area affected by any type of foliar fungal disease at R5 (kernel dent stage). Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year					
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12	SEPAC '13
	% Severity on earleaf at R5					
Control	0.8 ab†	0.9 ab	7.5	1.0 ab	0.1	0.25 a
V5-C	0.9 a	1.0 a	7.2	1.0 a	0.1	0.13 bc
V5-H	0.7 bc	0.9 a	8.1	0.8 bc	0.2	0.17 ab
V5-Q	0.7 bc	0.9 ab	7.3	0.8 bc	0.0	0.15 bc
V5-S	0.6 c	0.8 b	7.6	0.7 cd	0.0	0.15 bc
V5R1-H	0.3 d	0.6 c	6.3	0.6 e	0.0	0.09 c
R1-H	0.3 d	0.6 c	6.1	0.7 ed	0.0	0.08 c
Grand Mean			7.2		0.1	
Level of Significance	**≤0.0001**	**≤0.0001**	0.1	**≤0.0001**	0.3	**0.009**
Contrasts‡	Level of Significance					
Control vs. V5	0.06	0.6	0.8	**0.01**	0.9	**0.01**
Control vs. R1	**≤0.0001**	**≤0.0001**	**0.05**	**≤0.0001**	0.08	**0.0002**
V5 vs. R1	**≤0.0001**	**≤0.0001**	**0.006**	**≤0.0001**	**0.04**	**0.006**
Control vs. V5-C	0.2	0.1	0.7	0.5	0.8	**0.007**
V5-H vs. V5R1-H	**≤0.0001**	**≤0.0001**	**0.03**	**≤0.0001**	**0.02**	**0.04**
R1-H vs. V5R1-H	0.5	1	0.7	0.1	0.5	0.7

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-16. Effect of foliar fungicides and a foliar fertilizer on SPAD values at R5 (kernel dent stage). The SPAD measurements were taken on the earleaf and the second leaf above the earleaf. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year										
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	SPAD Units, Earleaf					SPAD Units, Earleaf + 2					
Control	49.9 bcd†	53.2	49.6	51.6 b	45.8	45.5	54.3 abc	54.9	53.1 bc	53.0	51.6
V5-C	52.7 a	52.5	47.4	51.1 b	41.4	48.7	53.9 bc	54.3	51.1 c	53.3	46.9
V5-H	51.4 abc	53.2	49.0	51.8 ab	45.4	45.6	55.8 a	54.7	53.4 abc	53.4	50.7
V5-Q	52.0 ab	53.5	49.9	51.9 ab	43.0	45.5	56.0 a	54.0	54.4 ab	53.3	48.7
V5-S	49.5 cd	53.2	49.0	52.6 a	43.0	44.5	55.5 ab	53.7	54.3 ab	53.1	49.0
V5R1-H	48.7 d	52.7	51.4	51.8 ab	42.3	43.7	53.2 c	53.6	55.2 ab	52.9	48.8
R1-H	49.8 bcd	54.0	51.4	51.4 b	41.7	43.6	54.6 abc	54.8	55.6 a	53.1	48.5
Grand Mean		53.2	49.7		43.2	45.3		54.3		53.1	49.2
Level of Significance	**0.009**	0.8	0.07	**0.03**	0.2	0.1	**0.03**	0.8	**0.01**	0.9	0.07
LSD (0.05)	2.3	ns	ns	0.8	ns	ns	1.8	ns	2.3	ns	ns
C.V.	5	3	4	2	8	5	2	3	3	2	5
Contrasts‡	Level of Significance										
Control vs. V5	0.3	0.9	0.8	0.2	0.2	0.8	**0.04**	0.4	0.3	0.5	0.1
Control vs. R1	0.5	0.8	0.1	0.9	**0.03**	0.2	0.6	0.5	**0.03**	0.9	**0.03**
V5 vs. R1	**0.03**	0.9	**0.02**	0.06	0.2	0.2	**0.003**	0.9	0.07	0.4	0.4
Control vs. V5-C	**0.02**	0.5	0.1	0.2	**0.03**	0.09	0.6	0.6	0.1	0.5	**0.004**
V5-H vs. V5R1-H	**0.03**	0.7	0.07	1.0	0.1	0.3	**0.01**	0.3	0.1	0.2	0.2
R1-H vs. V5R1-H	0.4	0.3	1.0	0.3	0.7	1.0	0.1	0.3	0.8	0.6	0.8

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-17. Effect of foliar fungicides and a foliar fertilizer on the number of green leaves present below the earleaf at R5 (kernel dent stage). The portion of a leaf still green was recorded as 0, 1/4, 1/2, 3/4, or 1, and the values were summed to give the number of green leaves per plant. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year				
	ACRE	DPAC	NEPAC	PPAC	SEPAC
Treatment	'12 & '13	'13	'13	'12 & '13	'12 & '13
	# Green Leaves at R5				
Control	3.8	4.5	1.3	3.8 c†	1.6 abc
V5-C	4.1	4.4	1.6	3.8 c	1.4 c
V5-H	4.1	4.8	1.4	4.2 ab	1.9 a
V5-Q	4.1	4.5	1.5	4.0 b	1.5 bc
V5-S	3.7	4.7	2.1	4.2 ab	1.6 abc
V5R1-H	4.0	4.8	1.9	4.4 a	1.8 ab
R1-H	3.8	4.9	1.3	4.1 b	1.6 bc
Grand Mean	3.9	4.7	1.6		
Level of Significance	0.4	0.1	0.6	**≤0.0001**	**0.04**
LSD (0.05)	ns	ns	ns	0.2	0.3
C.V.	12	5	45	6	14
Contrasts‡	Level of Significance				
Control vs. V5	0.4	0.1	0.4	**0.0002**	0.5
Control vs. R1	0.6	**0.02**	0.5	**≤0.0001**	0.6
V5 vs. R1	0.8	0.2	0.9	0.3	1.0
Control vs. V5-C	0.2	0.6	0.6	0.9	0.2
V5-H vs. V5R1-H	0.7	1.0	0.3	0.2	0.4
R1-H vs. V5R1-H	0.4	0.7	0.2	**0.01**	0.2

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.7 Stalk Lodging at R6

Percent stalk lodging on the basis of a "push test" was lower at R6 (physiological maturity) in treatments with fungicides applied at R1 (3%) compared to the control (6%) or V5 foliar fungicides (5%) at PPAC (2-year mean) otherwise fungicide treatments had no effect on stalk strength in the other trials (Control vs. R1 and V5 vs. R1 contrasts; Table 2-18). Reduced lodging at PPAC may be related to the increased number of green leaves (Table 2-17) and lower disease severity (Table 2-15) with the R1 treatments at R5 and thus an increase in photosynthetic leaf area below the earleaf. Plants with less photosynthetically active leaf area during the grain filling period are more susceptible to stalk cannibalization than plants having more photosynthetically active leaf area (Dodd, 1980). Stalk integrity, assessed on the University of Illinois disease severity scale where 0 is no discoloration and 5 is lodging, was improved by the application of pyraclostrobin at R1 under low levels of disease (≤ 5% severity on the earleaf at R4) at two of four locations in Iowa (Byamukama et al., 2013).

Table 2-18. Effect of foliar fungicides and a foliar fertilizer on the percentage of stalk lodging at R6 (physiological maturity). Plants were considered lodged when plants did not return to upright position after being pushed 30 cm horizontally at ear height. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year				
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	% Lodged				
Control	5†	16	47	6	58
V5-C	11	21	49	5	63
V5-H	2	9	44	5	62
V5-Q	4	20	40	5	63
V5-S	11	14	49	4	59
V5R1-H	1	18	33	2	56
R1-H	4	8	37	3	53
Grand Mean	6	15	43	4	59
Level of Significance	0.06	0.7	0.8	0.2	0.4
Contrasts‡	Level of Significance				
Control vs. V5	1.0	0.8	0.8	0.3	0.4
Control vs. R1	0.3	0.7	0.3	**0.02**	0.5
V5 vs. R1	0.2	0.8	0.3	**0.05**	0.06
Control vs. V5-C	0.2	0.6	0.9	0.7	0.3
V5-H vs. V5R1-H	0.7	0.4	0.4	0.1	0.3
R1-H vs. V5R1-H	0.4	0.2	0.7	0.7	0.5

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.8 Grain Yield and Yield Components

Foliar fungicides applied at V5 increased grain yields over the control by about 2% at DPAC in 2013, but had no effects in any other trials (Control vs. V5 contrast; Table 2-19). Grain yield was increased 2-4% by R1 applications of foliar fungicides at DPAC (2013), NEPAC (2013), and SEPAC (2-year mean) [Control vs. R1 contrasts; Table 2-19]. The likelihood of observing a yield response to foliar fungicides applied between VT and R1 is greater when hybrids are susceptible to disease, yields are < 9.1 Mg ha^{-1}, and disease severity is > 5% on the earleaf between R4 and R6 (Paul et al., 2011). Yield explained < 10% of the variability in yield response whereas disease severity explained a much greater portion of the responsiveness of grain yield to fungicides (Paul et al., 2011).

In this study, grain yield was approximately 2% greater with foliar fungicide applications at both V5 and R1 compared to the control at DPAC in 2013 (Table 2-19), but the hybrid was moderately resistant, yields averaged 11.4 Mg ha^{-1}, and disease severity at R5 was only 0.9% in the control (Table 2-15). At this particular location, there was no difference in yield response between the two foliar fungicide treatment timings (V5 vs. R1 contrast; Table 2-19). Disease severity and the number of leaves affected by disease were lower with the V5 foliar fungicide treatments compared to the control at V10 (Tables 2-4 and 2-5). A combination of early-season disease control, reduced stress, and fewer defense mechanisms induced by the plant (Bertelson et al., 2001) likely contributed to the increase in grain yield in plots treated with foliar fungicides at V5. In plots treated with foliar fungicides at R1, the combination of lower disease severity at R5 (Table 2-15), higher number of green leaves below the earleaf at R5 (Table 2-17), less stress, and fewer defense mechanisms induced by the plant likely

contributed to the increase in grain yield. Our findings contrast with the findings of Byamukama et al. (2013) who noted an increase in the proportion of green leaf area after an application of pyraclostrobin at R1, but no increase in grain yield was detected.

Grain yield was higher for R1 foliar fungicide treatments compared to the control at NEPAC in 2013 (Control vs. R1 contrast; Table 2-19). At this location, disease pressure was much greater than the other locations with disease severity being 7.5% for the control on the earleaf at R5 (Table 2-15) and the grain yield response to foliar fungicides was twice that of the other trials with significant effects (4% vs. 2%). In contrast to the DPAC trial, the R1 fungicide application timing was more effective than the V5 fungicide application timing at this location (V5 vs. R1 contrast; Table 2-19).

Grain yield at SEPAC (2-year mean) increased 4% in response to R1 foliar fungicide applications (Control vs. R1 contrast; Table 2-19) even though the hybrid grown at this location was rated highly resistant to foliar fungal diseases and disease severity averaged only 0.15% (Table 2-15). Grain yields were low at this location with an average of 7.4 Mg ha^{-1} (Table 2-19). The probability of obtaining a positive yield response to foliar fungicides was greater when yields were below 9.4 Mg ha^{-1} (Paul et al., 2011). The measured SPAD values were lower in the R1 foliar fungicide treatments than the control (Table 2-16), differences in disease severity between treated plots and the control were extremely small (<0.20 %; Table 2-15), green leaf numbers did not differ between treatments (Table 2-17), and there was no difference in stalk lodging (Table 2-18). The yield gain at this location cannot be adequately explained by any of the indicators of plant health measured in this experiment. This suggests that some type of physiological effect on this particular hybrid that was not measured may have contributed

to the 2% yield increase in plots treated with foliar fungicides at R1 compared to the control.

Foliar fungicides applied at V5 increased grain moisture content at harvest at PPAC (2-year mean) only (Control vs. V5 contrast; Table 2-20). Grain moisture was slightly higher at harvest for the R1 foliar fungicide treatments than the control at DPAC (2013), NEPAC (2013), and PPAC (2-year mean) [Control vs. R1 contrasts; Table 2-20]. Differences in grain moisture between the treated plots and the control were 0.3, 0.5, and 0.2 percentage points at DPAC, NEPAC, and PPAC, respectively. The increase in grain moisture at harvest might be due to delayed senescence (higher percentage of green leaves and photosynthetically active plant material at R5) in the treated plots. However, in another study on corn, pyraclostrobin had no effect on grain moisture even though treated plants retained a green canopy for a longer period of time (Byamukama et al., 2013).

Relative to the control, none of the V5 or R1 foliar fungicide treatments had significant effects on individual yield components, except for thousand-kernel weight at NEPAC in 2013 (Control vs. V5 and Control vs. R1 contrasts) [Tables 2-21 & 2-22]. Thousand-kernel weight for the R1 foliar fungicide treatments averaged 5% greater at NEPAC (Table 2-22). The increase in grain yield at several locations without detectable changes in individual yield components is most likely the result of small sub-samples within a large field-scale experiment and the small differences that would need to be detected in the individual ears collected. For example, the average yield increase observed between DPAC, NEPAC, and SEPAC was 330 kg ha^{-1} and the average kernel weight was 0.27 g $kernel^{-1}$. At 81,500 plants ha^{1}, it only takes an additional kernel row^{-1}

on each ear on a 16-row ear to achieve the observed increase in grain yield. See the calculation below:

$$\left(\frac{330\ kg}{1\ ha}\right)\left(\frac{1\ ha}{81500\ ears}\right)\left(\frac{1\ kernel}{0.00027\ kg}\right)\left(\frac{1\ row}{16\ kernels}\right) = 0.94\ kernels/row$$

Table 2-19. Effect of foliar fungicides and a foliar fertilizer on grain yield. Grain yield was estimated with a calibrated yield monitor in a commercial harvester. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year									
	ACRE	DPAC		NEPAC		PPAC		SEPAC		
Treatment	'12 & '13	'13		'13		'12 & '13		'12 & '13		
	Mg ha^{-1}									
Control	9.8	11.3	b†	11.5	bc	13.0	bcd	7.3	bc	
V5-C	9.9	11.3	b	11.3	c	13.0	d	7.2	c	
V5-H	9.8	11.7	a	11.4	bc	13.2	ab	7.4	bc	
V5-Q	9.7	11.5	ab	11.4	bc	13.0	cd	7.4	bc	
V5-S	9.7	11.4	b	11.5	bc	13.2	abc	7.5	ab	
V5R1-H	9.8	11.4	ab	11.9	ab	13.1	bcd	7.7	a	
R1-H	9.8	11.6	a	12.1	a	13.3	a	7.5	abc	
Grand Mean	9.8									
Level of Significance	0.9	**0.03**		**0.02**		**0.003**		**0.04**		
LSD (0.05)	ns	0.2		0.5		0.2		0.3		
C.V.	4	1		3		1		3		
Contrasts‡	Level of Significance									
Control vs. V5	0.6	**0.04**		0.7		0.4		0.3		
Control vs. R1	1.0	**0.03**		**0.02**		0.06		**0.04**		
V5 vs. R1	0.6	0.7		**0.002**		0.1		0.1		
Control vs. V5-C	0.6	1.0		0.3		0.3		0.5		
V5-H vs. V5R1-H	0.9	0.06		**0.05**		0.3		**0.04**		
R1-H vs. V5R1-H	1.0	0.1		0.4		**0.01**		0.07		

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-20. Effect of foliar fungicides and a foliar fertilizer on grain moisture at R6 (physiological maturity). Grain moisture was estimated with a calibrated yield monitor in a commercial harvester. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year				
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	% Moisture				
Control	19.3 b†	17.9	19.7 b	19.3 c	19.5
V5-C	19.0 c	18.0	19.7 b	19.3 bc	19.6
V5-H	19.2 b	18.1	19.7 b	19.5 a	19.4
V5-Q	19.3 ab	18.0	19.8 b	19.5 a	19.6
V5-S	18.9 c	18.0	19.7 b	19.5 ab	19.6
V5R1-H	19.5 a	18.2	20.2 a	19.5 a	19.8
R1-H	19.2 b	18.1	20.2 a	19.4 abc	19.7
Grand Mean		18.0			19.6
Level of Significance	**≤0.0001**	0.3	**0.009**	**0.0008**	0.7
LSD (0.05)	0.2	ns	0.3	0.1	ns
C.V.	1	1	1	1	2
Contrasts‡	Level of Significance				
Control vs. V5	0.3	0.2	0.9	**≤0.0001**	0.9
Control vs. R1	0.3	**0.03**	**0.003**	**0.001**	0.2
V5 vs. R1	**0.01**	0.1	**0.0003**	0.3	0.1
Control vs. V5-C	**0.01**	0.4	0.9	0.2	0.8
V5-H vs. V5R1-H	**0.01**	0.5	**0.009**	1.0	0.1
R1-H vs. V5R1-H	**0.01**	0.5	1.0	0.06	0.9

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-21. Effect of foliar fungicides and a foliar fertilizer on kernel rows ear^{-1} and kernels row^{-1} at R6 (physiological maturity). Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year										
Treatment	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	Rows Ear^{-1}						Kernels Row^{-1}				
Control	13.2†	15.3	14.9	15.7	15.8	16.2	32	36	34	35	29
V5-C	13.6	15.7	15.1	15.6	16.0	16.2	33	36	34	35	29
V5-H	12.9	15.7	15.3	15.6	15.9	16.2	32	36	34	34	30
V5-Q	13.2	15.7	15.5	15.7	15.7	16.3	33	36	35	35	29
V5-S	13.3	15.8	14.9	15.6	16.0	16.0	32	35	35	34	29
V5R1-H	12.1	15.2	14.9	15.5	15.8	16.3	32	36	35	35	30
R1-H	13.7	15.6	15.1	15.7	15.8	16.3	32	37	35	35	29
Grand Mean	13.1	15.6	15.1	15.6	15.8	16.2	32	36	35	35	29
Level of Significance	0.2	0.2	0.7	1.0	0.3	0.6	0.3	0.8	0.9	0.1	0.3
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C.V.	6	2	6	2	2	2	3	3	3	2	4
Contrasts‡	Level of Significance										
Control vs. V5	0.9	0.06	0.3	0.9	0.5	0.4	0.7	0.6	0.4	0.3	0.9
Control vs. R1	0.6	0.8	0.8	0.9	0.6	0.9	0.5	0.8	0.2	0.5	0.5
V5 vs. R1	0.6	**0.04**	0.4	1.0	0.9	0.2	0.2	0.7	**0.002**	0.7	0.3
Control vs. V5-C	0.4	0.1	0.6	0.9	0.08	0.7	0.4	0.6	0.3	0.6	0.9
V5-H vs. V5R1-H	0.2	0.06	0.3	0.7	0.7	0.5	0.8	0.8	**0.02**	0.3	0.9
R1-H vs. V5R1-H	**0.01**	0.1	0.6	0.4	0.9	1.0	0.3	0.4	0.3	0.7	0.2

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table 2-22. Effect of foliar fungicides and a foliar fertilizer on kernels ear^{-1} and thousand-kernel weight at R6 (physiological maturity). Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

	Location and Year									
Treatment	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	Kernels Ear^{-1}					Thousand Kernel Weight (g)				
Control	472	544	535	549	459	309 bc†	299	264	319	246
V5-C	491	542	524	561	451	311 bc	290	265	320	235
V5-H	470	551	525	545	463	319 a	297	263	323	247
V5-Q	487	564	549	551	436	314 ab	289	274	323	236
V5-S	478	529	550	546	435	304 c	292	277	321	244
V5R1-H	460	538	550	550	461	312 abc	304	278	322	243
R1-H	475	558	544	547	428	312 ab	305	275	324	243
Grand Mean	476	547	540	550	448	311	297	271	322	242
Level of Significance	0.1	0.7	0.2	0.5	0.3	**0.02**	0.2	0.1	0.4	0.3
LSD (0.05)	ns	ns	ns	ns	ns	8	ns	ns	ns	ns
C.V.	1	5	3	1	2	2	3	3	2	4
Contrasts‡	Level of Significance									
Control vs. V5	0.4	0.8	0.5	0.8	0.4	0.3	0.2	0.1	0.1	0.4
Control vs. R1	0.6	0.8	0.3	0.9	0.4	0.4	0.4	**0.02**	0.06	0.6
V5 vs. R1	0.1	1.0	0.5	0.9	1.0	1.0	**0.02**	0.2	0.6	0.8
Control vs. V5-C	0.06	0.9	0.4	0.1	0.7	0.7	0.2	0.9	0.5	0.06
V5-H vs. V5R1-H	0.3	0.5	0.07	0.5	0.9	0.07	0.3	**0.02**	0.7	0.5
R1-H vs. V5R1-H	0.1	0.4	0.6	0.7	0.1	0.8	0.9	0.6	0.3	0.9

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.9 Grain Nutrient Analysis

Treatments had very little effect on grain nutrient concentration (Tables A1-A5). Fungicides applied at R1 increased 2-year mean grain N concentration (Control vs. R1 contrast; Table 2-23) from 12.0 to 12.4 and 12.5 to 13.1 g kg^{-1} at PPAC and SEPAC, respectively. Grain N concentration was not affected by fungicide treatments at any other location. Few effects were observed on any other macronutrients (Tables A1-A5). Where differences did exist, a magnitude of approximately 0.2 g kg^{-1} was detected. Treatments also had very little to no effect on micronutrients (Tables A1-A5). In the few cases where differences were observed, a magnitude of approximately 1 mg kg^{-1} was noted.

Table 2-23. Effect of fungicides and a foliar fertilizer on grain N concentration at R6 (physiological maturity). Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means averaged over two years at one location are denoted by '12 & '13.

Treatment	Location and Year				
	ACRE '12 & '13	DPAC '13	NEPAC '13	PPAC '12 & '13	SEPAC '12 & '13
	g N kg^{-1}				
Control	11.8†	12.0	11.9	12.0	12.5
V5-C	11.9	11.8	11.3	12.3	13.1
V5-H	11.6	11.4	12.1	12.3	12.7
V5-Q	12.1	12.0	11.4	12.1	13.0
V5-S	11.8	11.9	12.0	12.4	12.9
V5R1-H	12.1	11.8	12.2	12.4	13.0
R1-H	11.8	12.0	11.5	12.3	13.2
Grand Mean	11.9	11.8	11.8	12.3	12.9
Level of Significance	0.3	0.6	0.2	0.2	0.2
LSD (0.05)	ns	ns	ns	ns	ns
C.V.	3	3	5	3	3
Contrasts‡	Level of Significance				
Control vs. V5	1.0	0.4	0.9	0.1	0.1
Control vs. R1	0.5	0.7	0.9	**0.02**	**0.02**
V5 vs. R1	0.4	0.5	0.9	0.2	0.2
Control vs. V5-C	0.8	0.5	0.2	0.1	**0.02**
V5-H vs. V5R1-H	**0.03**	0.2	0.9	0.5	0.3
R1-H vs. V5R1-H	0.3	0.7	0.1	0.7	0.4

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

2.3.10 Effects of Foliar Fertilizer (V5-C)

The foliar fertilizer (V5-C) treatment had little effect throughout the growing season at all of the locations. No effect of treatment was observed on leaf canopy

temperature measured approximately 1.5 to 2 weeks after the foliar fertilizer was applied (Table 2-10). No differences for leaf discoloration were observed between the control and foliar fertilizer (V5-C) treatments in 2012 (Table 2-11). At DPAC in 2013, treatments (0.35%) reduced disease severity compared to the control (0.54%; Table 2-11). Fewer diseased leaves were also observed with the foliar fertilizer (V5-C) treatment (1.4) than with the control (2.8) at DPAC in 2013 (Table 2-12). A study on winter wheat demonstrated increased severity of fungal diseases with increasing N rates, and authors suggested that pathogens are strongly affected by the N nutrition of the host (Olesen et al., 2003). Foliar applications of B to durum wheat decreased the severity of a fungal disease, and the authors suggested that the reduced severity was likely due to the involvement of B in physiology and biochemistry of the plant (Simoglou and Dordas, 2006).

No differences in crop canopy reflectance were observed at V10 in response to foliar fertilizer (V5-C) treatments at any of the locations (Table 2-9). This suggests that the foliar fertilizer applied at V5 had no effect on LAI, biomass, or chlorophyll status at V10. Although the V5-C treatment reduced levels of disease up to the V10 sample timing (Tables 2-4 and 2-5) at DPAC, no differences in disease were observed at R1 at any location (Table 2-13). No differences were observed for earleaf SPAD values at R1 (Table 2-14), suggesting that the substitution of 11.2 kg of soil-applied sidedress N ha^{-1} with 11.2 kg of foliar-applied N ha^{-1} containing 0.2 kg B ha^{-1} had no effect on leaf chlorophyll status or N status as estimated by a SPAD chlorophyll meter.

At SEPAC in 2013, the V5-C treatment (0.13%) had lower disease severity than the control (0.25%) at R5 (Table 2-15). The V5-C treatment resulted in higher SPAD values at R5 on the earleaf compared to the control at ACRE (2-year mean), but lower

SPAD values compared to the control at SEPAC (2-year mean) [Table 2-16]. The foliar fertilizer (V5-C) treatment did not have consistent effects on SPAD values, suggesting that the treatment did not have any effect on leaf tissue chlorophyll status or N status at R5. The V5-C foliar fertilizer treatment had no effect on the number of green leaves present below the earleaf at R5 (Control vs. V5-C contrast; Table 2-17).

The foliar fertilizer treatment had no effect on stalk integrity (Table 2-18), grain yield (Table 2-19), or yield components (Tables 2-14 and 2-15) at R6 (physiological maturity). The V5-C foliar fertilizer treatment had no effect on grain moisture at any location except ACRE (2-year mean), where it was slightly lower than the control at harvest (Table 2-20). The V5-C treatment increased grain N concentration at SEPAC (2-year mean; Table 2-23), but no effects were noted at the other locations.

2.3.11 Economics

In a meta-analysis, "quantitative synthesis of research findings from multiple individual trials", on 212 fungicide trials, the probability of obtaining an economical return on fungicides applied from VT to R1 was greater when disease severity was > 5% in untreated plots between R4 and R6 (Paul et al., 2012). The authors considered multiple grain price and application cost combinations in the analysis with ranges of \$79 - 276 Mg^{-1} and \$40 – 95 ha^{-1}, respectively. They found the probability of losing money when applying fungicides to be > 50% for 85% of the grain price and application cost combinations when disease severity was < 5%. Conversely, when disease severity was > 5%, the probability of losing money was > 50% for only 33% of the grain price and application cost combinations.

In this study, disease severity was < 1% at R5 at all of the locations except NEPAC (2013), where disease severity had reached 7.5% by R5 (Table 2-15). No fungicide effect on yield was observed at ACRE or PPAC (2-year means; Table 2-15). Both V5 and R1 applications of fungicides increased grain yields by 0.2 Mg ha^{-1} at DPAC (2013; Table 2-15). Fungicides applied at R1 increased grain yields at NEPAC (2013) by 0.5 Mg ha^{-1} and at SEPAC (2-year mean) by 0.3 Mg ha^{-1} (Table 2-15). At current grain prices of approximately \$190 Mg^{-1} and average cost of \$57.31 ha^{-1} to apply the fungicides in this study, a yield increase of approximately 0.3 Mg ha^{-1} was required to offset the cost of applying these treatments. Based on these figures, applying fungicides was only profitable at NEPAC in 2013, where disease severity was > 5% at R5. The chance of offsetting the cost of applying foliar fungicides is greater when grain prices are high and application costs are low.

Substituting 11.2 kg of soil-applied sidedress N ha^{-1} with 11.2 kg of foliar-applied N ha^{-1} containing 0.2 kg B ha^{-1} had no effect on grain yield (Table 2-15). The average price of soil-applied N fertilizer is approximately \$1.1 kg^{-1} N (J.J. Camberato, personal communication), and the foliar fertilizer used in this study cost \$9.7 kg^{-1} N with an average application cost of \$17.3 ha^{-1}. At current grain prices of \$190 Mg^{-1}, a yield increase of 0.05 Mg ha^{-1} would be required to offset the cost of applying this foliar fertilizer (\$9.7 kg^{-1} N) compared to traditional soil-applied N (\$1.1 kg^{-1} N). Since no yield response was observed at any of the locations, this was not a profitable practice.

2.3.12 Conclusion

A summary of the "Control vs. V5" and "Control vs. R1" contrasts may be found in Tables A-6 and A-7. Foliar fungicides had inconsistent effects on the majority of the parameters of plant health and grain yield across 8 trials. The most consistent effect of fungicides on plant parameters across locations was disease control. Applying fungicides at V5 reduced disease severity and progression until approximately V10 at DPAC (2013), NEPAC (2013), and SEPAC (2-year mean); however, by R1, no difference in disease was observed at these particular locations because of the absence of disease control or environmental conditions that were not conducive for disease development. At PPAC in 2013, the targeted V5 application of fungicides (actually applied at V7) reduced the incidence of disease at R1 and reduced earleaf disease severity at R5. Foliar fungicide applications at R1 reduced disease severity at R5 at all locations. The effectiveness of foliar fungicides on disease control is largely influenced by the timing of application and the onset of foliar diseases. Overall, the R1 fungicide application timing was more effective at keeping disease levels to a minimum during grain fill and ultimately resulted in lower disease severity at R5 as compared to the V5 fungicide application timing.

Applying fungicides at V5 had no effect on crop canopy reflectance, leaf surface temperatures, or R1 SPAD values. Fungicides applied at R1 increased SPAD values at NEPAC (2013), decreased SPAD values at SEPAC (2-year mean), and had no effect on SPAD values at ACRE, DPAC, or PPAC. Applying fungicides at R1 increased the amount of green leaf area present below the earleaf at R5 at DPAC, and both the V5 and R1 fungicide applications increased the amount of green leaf area at PPAC (2-year mean). Stalk strength was only increased at one of five locations (PPAC, 2-year mean) in

response to an R1 application of foliar fungicides. Very few and inconsistent effects on the parameters of plant health were observed in response to foliar fungicides, but more effects were observed in response to the R1 fungicide application timing than the V5 application timing.

Out of the 8 trials in this study, fungicides applied at V5 only increased grain yield in 1 trial (DPAC in 2013) or 13% of the time, and foliar fungicide applications at R1 increased grain yield in 4 of 8 trials or 50% of the time. Fungicides applied at R1 increased grain yield at DPAC (2013), NEPAC (2013), and SEPAC (2-year mean). Disease severity was only greater than 5% on the earleaf at R5 in 1 trial (NEPAC in 2013), and this location had the largest yield increase in response to foliar fungicide applications made at R1. When disease severity was less than 5% on the earleaf, there was a positive yield response to fungicides applied at R1 in only 3 trials or 38% of the time. Of the 4 trials that had near 100% incidence of disease at R1 (ACRE, DPAC, NEPAC, and PPAC in 2013), 2 of the 4 had a positive yield response to fungicides applied at R1. For the 8 trials in this study, the chances of having a positive yield response to fungicides was 50%, regardless of whether or not the field had a high incidence of disease at R1.

Based on these data, fungicides applied at V5 seldom improved the plant parameters measured and grain yield. Fungicides applied at R1 did not consistently improve the measured plant parameters or increase grain yield; however, chances of improving disease control, plant health, and grain yield were higher when applying fungicides at R1 as compared to V5. Applying fungicides at both V5 and R1 did not improve overall plant health or grain yields more than a single application at R1. Even in

the trials where a positive grain yield response was observed, obtaining a profitable return is dependent on application cost and grain prices. Chances of offsetting the application cost are greater when disease severity is > 5% at R5 (Paul et al., 2011); however, predicting the levels of expected disease severity proves to be difficult.

A summary of the "Control vs. V5-C" contrasts may be found in Table A-8. The foliar fertilizer (V5-C) treatment had essentially no effect on plant parameters measured at any of the locations. After applying the foliar fertilizer at V5, lower disease was observed at V10 at DPAC in 2013 and at R5 at SEPAC (2-year mean), but no effect was observed at the other locations. Applying the foliar fertilizer had no effect on SPAD values at R1, and effects on SPAD values at R5 were inconsistent. The foliar fertilizer increased grain N concentration 0.6 g N kg^{-1} at SEPAC (2-year mean), but it had no effect at the other locations. Overall, the foliar fertilizer treatment had almost no effect on overall plant health, and where it did have an effect, it was inconsistent across locations. Grain yield was not higher than the control in response to the foliar fertilizer treatment at any of the locations.

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APPENDIX

Table A-1. Effect of fungicides and a foliar fertilizer on grain nutrient concentration at R6 (physiological maturity) at ACRE averaged over two growing seasons. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means that are not averaged over two years are denoted by the year.

						Nutrient				
	N	P '12†	P '13†	K	S	Mg	Mn '12†	Mn '13†	Zn	Fe
Treatment	---------- g kg^{-1} ----------						---------- mg kg^{-1} ----------			
Control	11.8	2.2 b‡	2.2	3.9	1.0	1.0	4	4	17	14
V5-C	11.9	2.2 b	2.3	4.0	1.0	1.0	5	4	17	15
V5-H	11.6	2.6 a	2.4	4.2	1.0	1.1	5	4	18	16
V5-Q	12.1	2.5 a	2.6	4.3	1.0	1.1	5	4	19	17
V5-S	11.8	2.4 ab	2.3	4.2	1.0	1.1	5	3	17	16
V5R1-H	12.1	2.4 ab	2.2	4.0	1.0	1.1	5	3	17	15
R1-H	11.8	2.5 a	2.4	4.3	1.0	1.1	5	5	18	16
Grand Mean	11.9	2.4	2.3	4.1	1.0	1.1	5	4	18	15
Level of Significance	0.3	**0.03**	0.6	0.09	0.06	0.2	0.4	0.4	0.08	0.2
LSD (0.05)	ns	0.02	ns	ns	ns	ns	ns	ns	ns	ns
C.V.	3	7	14	8	5	10	14	36	10	14
Contrasts§	---------- Level of Significance ----------									
Control vs. V5	1.0	**0.009**	0.3	**0.01**	**0.02**	**0.02**	**0.05**	0.7	0.06	**0.02**
Control vs. R1	0.5	**0.03**	0.8	0.1	0.2	**0.05**	0.07	0.9	0.4	0.07
V5 vs. R1	0.4	0.6	0.4	0.3	0.3	0.8	1	0.7	0.2	0.5
Control vs. V5-C	0.8	1	0.9	0.6	0.3	0.4	0.3	0.8	0.9	0.5
V5-H vs. V5R1-H	**0.03**	0.09	0.3	0.2	**0.02**	0.5	0.6	0.3	**0.05**	0.7
R1-H vs. V5R1-H	0.3	0.2	0.4	0.1	**0.02**	0.2	1	**0.02**	0.06	0.6

† Represents data from one growing season only.

‡ Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

§ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

¶ See Table 2-4 for active ingredients and application rates of treatments.

Table A-2. Effect of fungicides and a foliar fertilizer on grain nutrient concentration at R6 (physiological maturity) at DPAC in 2013. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H).

	Nutrient							
	N	P	K	S	Mg	Mn	Zn	Fe
Treatment	---------- g kg^{-1} ----------					---------- mg kg^{-1} ----------		
Control	12.0†	2.9	4.7	0.9	1.1	4	17	16
V5-C	11.8	3.1	4.8	1.0	1.1	4	18	17
V5-H	11.4	2.8	4.6	1.0	1.1	4	17	17
V5-Q	12.0	2.9	4.6	1.0	1.1	5	18	17
V5-S	11.9	3.2	4.9	1.0	1.2	4	19	18
V5R1-H	11.8	3.1	4.9	0.9	1.2	5	19	17
R1-H	12.0	3.0	4.9	1.0	1.1	4	19	17
Grand Mean	11.8	3.0	4.8	1.0	1.1	4	18	17
Level of Significance	0.6	0.5	0.2	0.2	0.7	0.9	0.6	0.6
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns
C.V.	3	8	4	4	9	24	8	9
Contrasts‡	---------- Level of Significance ----------							
Control vs. V5	0.4	0.4	0.9	0.1	0.4	0.7	0.4	0.08
Control vs. R1	0.7	0.3	0.1	0.2	0.3	1.0	0.2	0.3
V5 vs. R1	0.5	0.6	0.08	0.6	0.8	0.7	0.5	0.4
Control vs. V5-C	0.5	0.3	0.7	0.06	0.5	0.7	0.4	0.2
V5-H vs. V5R1-H	0.2	0.2	0.1	0.3	0.3	0.4	0.2	0.6
R1-H vs. V5R1-H	0.7	0.7	0.8	0.06	0.7	0.4	0.8	0.8

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table A-3. Effect of fungicides and a foliar fertilizer on grain nutrient concentration at R6 (physiological maturity) at NEPAC in 2013. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H).

	Nutrient							
	N	P	K	S	Mg	Mn	Zn	Fe
Treatment	---------- g kg^{-1} ----------					---------- mg kg^{-1} ----------		
Control	11.9†	2.8	4.6	1.1	1.1	5	18	15
V5-C	11.3	2.8	4.5	1.0	1.0	4	17	15
V5-H	12.1	2.5	4.1	1.0	0.9	4	16	15
V5-Q	11.4	2.9	4.7	1.1	1.0	5	17	15
V5-S	12.0	2.8	4.4	1.0	1.0	4	17	15
V5R1-H	12.2	2.9	4.7	1.1	1.1	5	18	15
R1-H	11.5	2.7	4.5	1.0	1.0	4	16	14
Grand Mean	11.8	2.8	4.5	1.0	1.0	4	17	15
Level of Significance	0.2	0.3	0.1	1.0	0.5	0.6	0.06	0.9
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns
C.V.	5	7	6	5	10	31	6	11
Contrasts‡	---------- Level of Significance ----------							
Control vs. V5	0.9	0.4	0.3	0.1	0.2	0.3	**0.03**	0.7
Control vs. R1	0.9	0.8	0.9	0.2	1.0	0.7	0.07	0.9
V5 vs. R1	0.9	0.6	0.19	0.6	0.1	0.4	0.6	0.5
Control vs. V5-C	0.2	0.7	0.7	0.06	0.7	0.2	0.1	0.7
V5-H vs. V5R1-H	0.9	**0.03**	**0.009**	0.3	**0.04**	0.1	**0.02**	1.0
R1-H vs. V5R1-H	0.1	0.2	0.2	0.06	0.5	0.2	0.06	0.3

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table A-4. Effect of fungicides and a foliar fertilizer on grain nutrient concentration at R6 (physiological maturity) at PPAC averaged over two growing seasons. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H). Means that are not averaged over two years are denoted by the year.

	Nutrient								
	N	P	K	S	Mg	Mn '12†	Mn '13†	Zn	Fe
Treatment	g kg^{-1}					mg kg^{-1}			
Control	12.0‡	3.0	4.3	0.8	1.2	5	4	21	18
V5-C	12.3	3.0	4.3	0.8	1.2	5	6	20	16
V5-H	12.3	2.9	4.2	0.8	1.1	4	4	20	16
V5-Q	12.1	2.9	4.2	0.8	1.2	5	6	20	17
V5-S	12.4	2.9	4.3	0.8	1.2	5	6	20	16
V5R1-H	12.4	3.0	4.3	0.8	1.2	5	7	21	17
R1-H	12.3	3.0	4.4	0.8	1.2	5	5	20	16
Grand Mean	12.3	3.0	4.3	0.8	1.2	5	5	20	17
Level of Significance	0.2	0.8	0.8	0.6	0.9	1.0	0.1	0.8	0.4
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns
C.V.	3	9	6	7	9	15	28	8	11
Contrasts§	Level of Significance								
Control vs. V5	0.1	0.3	0.4	0.6	0.3	0.5	0.3	0.2	0.08
Control vs. R1	**0.02**	0.9	1.0	0.6	0.9	0.6	0.1	0.7	0.3
V5 vs. R1	0.2	0.3	0.3	0.2	0.2	0.8	0.4	0.2	0.5
Control vs. V5-C	0.1	0.9	0.9	0.7	0.8	1.0	0.2	0.4	0.1
V5-H vs. V5R1-H	0.5	0.7	0.5	0.4	0.2	0.7	**0.01**	0.2	0.2
R1-H vs. V5R1-H	0.7	0.9	0.7	0.4	0.8	1.0	0.1	0.6	0.3

† Represents data from one growing season only.

‡ Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

§ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

¶ See Table 2-4 for active ingredients and application rates of treatments.

Table A-5. Effect of fungicides and a foliar fertilizer on grain nutrient concentration at R6 (physiological maturity) at SEPAC averaged over two growing seasons. Treatments were applied at V5 as CoRoN (V5-C), Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S). Treatments were applied at R1 as Headline AMP (R1-H). One treatment received Headline at V5 and Headline AMP at R1 (V5R1-H).

	Nutrient							
	N	P	K	S	Mg	Mn	Zn	Fe
Treatment	---------- g kg^{-1} ----------					---------- mg kg^{-1} ----------		
Control	12.5	2.8	4.4	1.0	1.2	6 b†	20	19
V5-C	13.1	3.0	4.6	1.1	1.3	8 a	21	20
V5-H	12.7	2.9	4.5	1.0	1.2	7 a	20	20
V5-Q	13.0	3.1	4.7	1.0	1.3	8 a	21	21
V5-S	12.9	3.0	4.5	1.0	1.3	7 a	23	20
V5R1-H	13.0	3.0	4.6	1.1	1.3	8 a	21	20
R1-H	13.2	2.9	4.5	1.0	1.2	7 a	20	20
Grand Mean	12.9	3.0	4.5	1.03	1.26	7	21	20
Level of Significance	0.2	0.4	0.5	0.7	0.1	**0.01**	0.3	0.5
LSD (0.05)	ns	ns	ns	ns	ns	0.77	ns	ns
C.V.	3	8	5	4	7	9	9	7
Contrasts‡	---------- Level of Significance ----------							
Control vs. V5	0.1	0.1	0.2	1.0	0.08	**0.0007**	0.3	0.2
Control vs. R1	**0.02**	0.2	0.2	0.7	0.3	**0.002**	0.9	0.7
V5 vs. R1	0.2	0.8	0.9	0.6	0.3	0.7	0.2	0.2
Control vs. V5-C	**0.02**	0.09	0.1	0.2	0.1	**0.001**	0.8	0.4
V5-H vs. V5R1-H	0.3	0.3	0.6	0.5	0.2	0.7	0.6	0.8
R1-H vs. V5R1-H	0.4	0.3	0.5	0.5	0.1	0.7	0.9	0.8

† Means without letters or followed by the same letter are not statistically different based on Fisher's protected LSD ($P \leq 0.05$).

‡ V5 consists of fungicide treatments V5-H, V5-Q, and V5-S and R1 consists of fungicide treatments V5R1-H and R1-H.

§ See Table 2-4 for active ingredients and application rates of treatments.

Table A-6. Summary of the "Control vs. V5" contrasts for all of the plant variables measured throughout the 2012 and 2013 growing seasons. The V5 fungicide application treatments consisted of Headline (V5-H), Quadris (V5-Q), and Stratego YLD (V5-S).

	Location and Year							
Assessment and Timing	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12	PPAC '13	SEPAC '12	SEPAC '13
Leaf Temperature (V7-V10)	n/a†	-‡	-	-	n/a	-	n/a	-
Disease Rating - Severity (V10)	-	-	*§	*	-	-	-	-
Disease Rating - Leaves Affected (V10)	-	-	*	*	-	-	-	*
Crop Reflectance (V10)	-	-	-	-	-	-	-	-
Disease Rating (R1)	-	-	-	-	-	*	-	-
SPAD Readings (R1)	-	-	-	-	-	-	-	-
Disease Rating (R5)	-	-	-	-	*		-	*
SPAD Readings - Earleaf (R5)	-	-	-	-	-	-	-	-
SPAD Readings - Earleaf + 2 (R5)	-	*	-	-	-	-	-	-
Green Leaf Rating (R5)	-	-	-	-	*		-	-
Push Test (Post R6)	-	-	-	-	-	-	-	-
Grain Yield	-	-	*	-	-	-	-	-
Grain Moisture	-	-	-	-	*		-	-
Grain N Concentration	-	-	-	-	-	-	-	-

† Not available because leaf surface temperatures were only collected during the 2013 growing season.
‡ Indicates a non-significant contrast ($P \leq 0.5$).
§ Indicates a significant contrast ($P \leq 0.5$).

Table A-7. Summary of the "Control vs. R1" contrasts for all of the plant variables measured throughout the 2012 and 2013 growing seasons. The R1 fungicide application treatments consisted of Headline AMP (R1-H) applied at R1 and a treatment receiving Headline at V5 and Headline AMP at R1 (V5R1-H).

	Location and Year							
Assessment and Timing	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12	PPAC '13	SEPAC '12	SEPAC '13
Disease Rating (R5)	*†		*	*		*	-‡	*
SPAD Readings - Earleaf (R5)	-	-	-	-	-	-	*	
SPAD Readings - Earleaf + 2 (R5)	-	-	-	*	-	-	*	
Green Leaf Rating (R5)	-	-	*	-	*		-	-
Push Test (Post R6)	-	-	-	-	*		-	-
Grain Yield	-	-	*	*	-	-	*	
Grain Moisture	-	-	*	*	*		-	-
Grain N Concentration	-	-	-	-	*		*	

† Indicates a significant contrast ($P \leq 0.5$).

‡ Indicates a non-significant contrast ($P \leq 0.5$).

Table A-8. Summary of the "Control vs. V5-C" contrasts for all of the plant variables measured throughout the 2012 and 2013 growing seasons. The V5-C treatment consisted of an application of the foliar fertilizer CoRoN at V5.

	Location and Year							
Assessment and Timing	ACRE '12	ACRE '13	DPAC '13	NEPAC '13	PPAC '12	PPAC '13	SEPAC '12	SEPAC '13
Leaf Temperature (Post V5)	n/a†	-‡	-	-	n/a	-	n/a	-
Disease Rating - Severity (Post V5)	-	-	*§	-	-	-	-	-
Disease Rating - Leaves Affected (Post V5)	-	-	*	-	-	-	-	-
Crop Reflectance (Post V5)	-	-	-	-	-	-	-	-
Disease Rating (R1)	-	-	-	-	-	-	-	-
SPAD Readings (R1)	-	-	-	-	-	-	-	-
Disease Rating (R5)	-	-	-	-	-	-	-	*
SPAD Readings - Earleaf (R5)		*	-	-	-	-	*	
SPAD Readings - Earleaf + 2 (R5)	-	-	-	-	-	-	*	
Green Leaf Rating (R5)	-	-	-	-	-	-	-	-
Push Test (Post R6)	-	-	-	-	-	-	-	-
Grain Yield	-	-	-	-	-	-	-	-
Grain Moisture		*	-	-	-	-	-	-
Grain N Concentration	-	-	-	-	-	-	*	

† Not available because leaf surface temperatures were only collected during the 2013 growing season.
‡ Indicates a non-significant contrast ($P \leq 0.5$).
§ Indicates a significant contrast ($P \leq 0.5$).